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Title: Metabolic Profiling to Determine Bactericidal or Bacteriostatic Effects of New Natural Products using Isothermal Microcalorimetry

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **Y**

Videographer: All screen captures provided, do not film

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **31**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Janetta Coetzee**: Understanding the modes of action of novel antibacterial compounds is important but challenging. This method is easy to implement and provides insight into these mechanisms **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Katarina Cirnski**: This technique allows the real-time observation of antibacterial effects in a non-invasive manner, enabling additional sample analyses **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Janetta Coetzee**: Although practice with microbiological techniques is a prerequisite, the experiment is easy to perform. Taking your time for data interpretation is also important when using microcalorimetry for the first time **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Sample Preparation

2.1. Begin by using a spectrophotometer at a wavelength of 600 nanometers to measure the optical density of the overnight culture **[1]**.

2.1.1. WIDE: Talent loading sample onto spectrophotometer

2.2. Dilute the culture to a 5 x 10^5 colony-forming units/milliliter of fresh MHB (M-H-B) medium concentration **[1-TXT]** and add 150 microliters of cells to each 1.5-milliliter tube of antibiotic at the concentration of interest **[2]**.

2.2.1. Talent adding cells to medium, with medium container visible in frame **TEXT: See text for all medium preparation details**

2.2.2. Talent adding cells to tube, with antibiotic container visible in frame **TEXT: OD_{600} of 1 = approximately 5 x 10^8 CFU/mL**

2.3. Then mix the compound with the cells by vortexing **[1]**.

2.3.1. Tube being vortexed

3. Insert Preparation and Loading

3.1. To prepare the inserts, add 120 microliters of each bacteria-antibiotic mixture into individual plastic inserts in the appropriate wells of a 48-well plate **[1]** and use tweezers to place all of the titanium vials into the holders **[2]**.

3.1.1. WIDE: Talent adding mixture to insert, with cell tube visible in frame *Videographer: Important/difficult step*

3.1.2. Talent placing vial into holder

3.2. Gently transfer the inserts into the titanium vials in the holder plate **[1]** and loosely place titanium lids onto all of the vials **[2]**.

3.2.1. Talent placing insert into holder plate *Videographer: Important step*

3.2.2. Talent placing lid onto vial

3.3. When all of the inserts have been transferred, place the holder onto the designated area on the sample station **[1]** and use a torque wrench set to 40 centinewton meters to tighten all of the lids **[2]**.

3.3.1. Talent placing holder
3.3.2. Lid(s) being tightened *Videographer: Important/difficult step*

4. Sample Analysis

4.1. In the system software, start a new experiment **[1]** and retract the sample insertion arm from the instrument **[2]**.

4.1.1. WIDE: Talent starting experiment, with monitor visible in frame
4.1.2. Talent retracting sample insertion arm

4.2. Place the cup holder on the "bridge" with column 8 facing the sample insertion opening **[1]** and gently push the cup holder into the instrument at "Position 1" **[2]**.

4.2.1. Talent placing cup holder onto bridge
4.2.2. Cup holder being pushed into position *Videographer: Important step*

4.3. Wait 10 minutes for the system to stabilize **[1]** before labeling the experimental wells **[2]**.

4.3.1. Talent setting timer or checking watch, with system visible in frame
4.3.2. Talent labeling wells, with monitor visible in frame

4.4. Next, push the sample insertion arm until the cup holder is at "Position 2" **[1]**. After allowing the system to stabilize for 20 minutes, push the sample insertion arm to "Position 3" **[2]** and retract the sample insertion arm until it is at the "Running position" **[3]**.

4.4.1. Talent pushing sample insertion arm to position 2 *Videographer: Important step*
4.4.2. Arm being pushed to position 3 *Videographer: Important step*
4.4.3. Arm being retracted

4.5. Highlight all of the wells in the software and select **Reaction Start [1]**.

4.5.1. SCREEN: screenshot_1: 00:00-00:10

4.6. Then run the experiment until the heat emission reads are stably back at zero. At the end of the analysis, select **Stop**. The software will then ask if "you are sure". Select **Yes** and save the experiment for data analysis **[1]**.

4.6.1. SCREEN: screenshot_2: 00:00-00:11

4.7. Then insert the sample insertion arm completely into the instrument **[1]** and engage the magnets to retrieve the cup holder **[2]**.

4.7.1. Talent inserting arm into instrument
4.7.2. Cup holder being retrieved with magnets

5. Data Analysis

5.1. To analyze the data, open the software **[1]** and select **Open experiment**. In the popup window, select the experiment of interest and click **Open [2]**.

5.1.1. WIDE: Talent opening software
5.1.2. SCREEN: screenshot_3 00:05-00:10

5.2. Click **Select all** and **Define Baseline** to normalize the data in each position **[1]**.

5.2.1. SCREEN: screenshot_3: 00:12-00:16

5.3. In the popup window, select a time period of greater than 30 minutes within the lag phase. After selection, the baseline will appear in green in the thermogram. Close the **Define Baseline Section** window **[1]**.

5.3.1. SCREEN: screenshot_3: 00:16-00:20

5.4. Then click **Save** and close the software **[1]**.

5.4.1. SCREEN: screenshot_3: 00:24-00:26

5.5. Next, open the web-based Symcel Calorimetry analysis application **[1-TXT]**.

5.5.1. Talent opening app **TEXT: https://symcel.shinyapps.io/symcel_calorimetricgrowth/**

5.6. Click **Browse** to upload the file of interest and select the experiment. The metabolic parameters will be automatically calculated for the 32 samples **[1]**.

5.6.1. SCREEN: screenshot_4: 00:02-00:15

5.7. To fit the heat Flow data to Gompertz and/or Richard´s growth models, click **Growth Function**. Growth models will be displayed in the “Cumulative” section for comparison to the raw data in the “Flow” section **[1]**.

5.7.1. SCREEN: screenshot_4: 00:16-00:27

5.8. To download the calculated parameters, click **Download Measures**, select the file location, and click **Save**. The file will be exported to a spreadsheet for further analysis **[1]**.

5.8.1. SCREEN: screenshot_4: 00:28-00:40

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
3.1.1., 3.2.1., 3.3.2., 4.2.2., 4.4.1., 4.4.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.1.1., 3.3.2.

Results

6. Results: Representative Antibiotic Effects on *A. baumannii* DSM-30008 Growth and Metabolism

6.1. Here the thermograms obtained by exposing *A. baumannii* DSM-30008 (D-S-M-three-zero-zero-zero-eight) to ciprofloxacin in serial dilution are displayed **[1]**.

6.1.1. LAB MEDIA: Figure 1A

6.2. Concentrations between 0.005- and 0.1-micromolar have a minimal effect on *A. baumannii* growth and metabolism **[1]**.

6.2.1. LAB MEDIA: Figure 1A *Video Editor: please emphasize 0.005 and 0.1 data lines*

6.3. Treating the cells with 0.5-micromolar ciprofloxacin, however, leads to a significant shift in the lag phase duration and to a lower maximum heat flow **[1]**.

6.3.1. LAB MEDIA: Figure 1A *Video Editor: please emphasize 0.5 data line*

6.4. These two changes together affect the time to peak **[1]**, resulting in an increase of approximately 6 hours **[2]**.

6.4.1. LAB MEDIA: Figure 1C *Video Editor: please emphasize 0.5 data bar*
6.4.2. LAB MEDIA: Figure 1C *Video Editor: please emphasize section of 0.5 data bar from tops of other data bars to top of 0.5 data bar*

6.5. In this figure, the cumulative released heat is plotted against time **[1]**, with the effect of each concentrations reflected by an incline of slope **[2]**.

6.5.1. LAB MEDIA: Figure 1B
6.5.2. LAB MEDIA: Figure 1B *Video Editor: please emphasize slope of 0.5 data line*

6.6. Quantification of the thermogram incline allows calculation of the maximum metabolic rate of *A. baumannii* in the presence of ciprofloxacin **[1]**, with a concomitant decrease in the metabolic rate observed in cells treated with 0.5-micromolar ciprofloxacin **[2]**.

6.6.1. LAB MEDIA: Figure 1D
6.6.2. LAB MEDIA: Figure 1D *Video Editor: please emphasize 0.5 data bar*

6.7. Rifampicin treatment has a dramatic effect on the thermograms of *A. baumannii* DSM-30008 **[1]**. A significant reduction of heat emission correlating with a decrease in metabolic activity is also observed **[2]**.

6.7.1. LAB MEDIA: Figures 4A and 4B *Video Editor: please emphasize Figure 4A*
6.7.2. LAB MEDIA: Figures 4A and 4B *Video Editor: please emphasize Figure 4B*

6.8. Note the increase in time to peak for all of the tested concentrations **[1]** and the decrease in the metabolic rate caused by the decrease in slope for all of the concentrations that are usually ascribed to a bactericidal effect **[2]**.

6.8.1. LAB MEDIA: Figures 4C and 4D *Video Editor: please emphasize 0.03125, 0.0625, and 0.125 data bars in Figure 4C*
6.8.2. LAB MEDIA: Figures 4C and 4D *Video Editor: please emphasize 0.03125, 0.0625, and 0.125 data bars in Figure 4D*

Conclusion

7. Conclusion Interview Statements

7.1. **Janetta Coetzee**: To ensure optimal results, use reverse pipetting and prevent additional fluid on the side of the insert when transferring samples to the inserts and close the lids properly **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1.-3.3.)