MIC Study on *Escherichia coli* ATCC 25922

LifeScale provides a rapid, quantitative and automated determination of antimicrobial susceptibility using a resonant mass method. As a highly sensitive liquid-based growth system, LifeScale lends itself to the direct measurement of liquid samples, such as clinical blood, CSF, and urine. LifeScale’s ability to count and weigh all of the individual microbes in a given sample volume ensures that growth is detected as early as possible, typically within the first few cell division cycles. Assuming a lag phase of approximately 40 minutes, during which time immature microbes are increasing in mass prior to cell division, and a cell division time of 20 minutes, LifeScale is able to detect an increase in microbe population in under 90 minutes.

LifeScale has demonstrated antimicrobial susceptibility test (AST) results consistent with reference methods for clinical isolates and quality control strains of Gram-negative and Gram-positive bacteria. Any combination of 20 antibiotics and/or antibiotic concentrations may be used with the LifeScale system.

Examples of LifeScale ASTs using breakpoint and minimal inhibitory concentration (MIC) approaches are described here.

**Breakpoints**

Samples taken directly from positive clinical blood cultures were tested against five different antibiotics at susceptible MIC (µg/ml) breakpoints. This small panel of antibiotics was selected after a Gram stain revealed Gram-positive bacteria in the sample. Blood samples required minimal processing, including centrifugation and dilution.

![Graph showing percentage growth of *Enterococcus faecalis* from clinical blood sample](image)

After two hours, LifeScale analysis clearly determined the susceptibility of the Gram-positive pathogen, which was later identified as *Enterococcus faecalis*. Susceptibility is easily discerned in the bar graph showing percentage growth over a two hour period.
The *Enterococcus faecalis* strain exhibited weak or no growth with ampicillin (8 µg/ml), ciprofloxacin (1 µg/ml) and trimethoprim-sulfamethoxazole [SXT 2/38 µg/ml], demonstrating susceptibility to these antibiotics at the specified concentrations. Conversely, the cefozolin (4 µg/ml) and gentamicin (4 µg/ml) treated samples exhibited over 400 percent growth, demonstrating resistance. A similar amount of growth is observed in the control. These AST results were later confirmed by the UCLA Clinical Microbiology Laboratory.

**Minimum inhibitory concentration**
LifeScale MIC results fall within MIC quality control ranges for Gram-negative and Gram-positive bacteria tested. As an example, the susceptibility of a quality control strain, *Escherichia coli* ATCC 25922, was treated with five different concentrations of imipenem. Samples were prepared using CLSI M07-A10 macrodilution method. Antibiotic concentrations included two-fold dilutions encompassing the MIC quality control range of 0.06 - 0.25 µg/ml (CLSI M100-S25 Table 5A). The resulting microbe/antibiotic combinations are shown in the table below.

<table>
<thead>
<tr>
<th>Vial</th>
<th>Microbe</th>
<th>Media</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Imipenem 0.03µg/ml</td>
</tr>
<tr>
<td>3</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Imipenem 0.06µg/ml</td>
</tr>
<tr>
<td>4</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Imipenem 0.125µg/ml</td>
</tr>
<tr>
<td>5</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Imipenem 0.25µg/ml</td>
</tr>
<tr>
<td>6</td>
<td>E. coli (ATCC 25922)</td>
<td>Luria Broth</td>
<td>Imipenem 0.5µg/ml</td>
</tr>
</tbody>
</table>

*Escherichia coli* ATCC 25922 Vs. imipenem microbe/antibiotic combinations

The control, imipenem [0.03 µg/ml], and imipenem [0.06 µg/ml] samples exhibit significant growth, as indicated in a plot of the absolute concentration in #/ml, below. Conversely, imipenem [0.125 µg/ml], imipenem [0.25 µg/ml], and imipenem [0.5 µg/ml] treated samples demonstrate negligible growth relative to the growth control, indicating susceptibility at these concentrations.

*Escherichia coli* ATCC 25922 Vs. imipenem concentration data measured by LifeScale.
After 3 hours the control sample, left, shows standard *E. coli* growth. The samples exposed to the antibiotic above the MIC show spheroplasts morphology changes.

Concentration data, #/ml, can also be visualized in the form of the percentage growth over the period of the measurement. Plotted in this way, the growth data indicates an increase in concentration in excess of 3,000%, below, for the control, imipenem (0.03 μg/ml), and imipenem (0.06 μg/ml) samples. Negligible growth is noted for the remaining samples in the same time period. The MIC then is 0.125 μg/ml, which falls within the acceptable MIC quality control range (imipenem 0.06 - 0.25 μg/ml) for *Escherichia coli* ATCC 25922.

Images, collected via video microscopy, indicate a morphology change for *Escherichia coli* exposed to imipenem at concentrations above the MIC. Representative images are shown below for the control as well as the 0.25 μg/ml imipenem sample at the three hour point. The control indicates a sample in the growth phase having a high concentration of rod-shaped *Escherichia coli*. In contrast the 0.25 μg/ml Imipenem sample shows a low concentration of microbes all of which have undergone a morphology change and present as spheroplasts. At this point remaining microbes are no longer viable.

The data clearly demonstrate results consistent with reference methods and indicate that breakpoint and MIC approaches work equally well in LifeScale AST analyses.

The MIC is easily discerned in a bar graph showing the percent growth over a three hour period.
A mechanically resonant structure, or resonator, such as a beam suspended at one end, resonates at a specific frequency, below. This frequency is known as the resonant frequency of the beam and is a function of its length and mass. When an additional mass is added to the beam, the resonant frequency decreases by an amount that is proportional to the added mass. Therefore, the additional mass can be calculated to very high precision by accurately measuring the shift in resonant frequency.

A resonant beam surrounded by water, or even air, dissipates a significant fraction of its vibrational energy to its environment. This energy loss has the effect of reducing the resolution to which changes in frequency, and hence mass, can be measured. To address this limitation, the resonator used in the instrument is enclosed in a vacuum environment allowing it to vibrate without dissipating energy unnecessarily. This yields a high Q or quality factor, typically greater than 10,000, allowing the resonant frequency to be measured to high precision.

LifeScale is able to measure frequency shifts of around 0.01Hz (20ppb) in a 1 kHz bandwidth. This measurement accuracy equates to a mass resolution of better than 1 femtogram and gives the mass of individual microbes to better than 1%.