

Don Whitley Scientific Limited develops, manufactures, and sells equipment and associated products for microbiology and cell culture applications worldwide. We also have an in-house microbiology laboratory, which began operation in the late 1980s. We offer a wide range of microbiological testing and have been GLP compliant since 1994. A particular area of expertise is antimicrobial susceptibility testing (AST), including the determination of Minimal Inhibitory Concentration (MIC) for antibacterial and antifungal agents.

As a manufacturer of anaerobic workstations, we offer MIC testing of fastidious anaerobic bacteria, including the normal human intestinal microbiota and, more recently, anaerobic bacteria that are being developed for use in live biotherapeutic products (LBPs). These are part of an emerging and diverse category of therapies containing live microorganisms used to treat, prevent, or cure diseases including gastrointestinal disorders, infectious disease, metabolic disorders and inflammatory conditions. Unlike probiotics, LBPs are regulated as medicinal products and require rigorous safety evaluation in GLP laboratory studies, which include screening of the constituent microbial strains for antimicrobial resistance.

At DWS, we use the agar dilution technique, as standardized by the Clinical & Laboratory Standards Institute (CLSI) to determine the MIC of antimicrobial agents against LBP candidate strains and other fastidious anaerobes. Using Whitley Anaerobic Workstations, we perform initial culture, inoculum preparation, MIC setup and plate incubation within a single, enclosed anaerobic environment (10% CO₂ / 10% H₂ / 80% N₂) without exposing the organisms to oxygen at any stage of the process. This is a specialized offering that distinguishes DWS from other contact microbiological testing laboratories.

Although agar dilution is the “gold standard” and the only method of MIC determination that can be applied to all the diverse species of anaerobic bacteria, it is labour-intensive to perform. For each agent to be tested, a series of twofold antimicrobial dilutions is prepared. Each dilution is added to a small volume of freshly prepared sterile, molten agar, which is then poured into a petri dish. After solidification (and, for anaerobic work, equilibration with the oxygen-free incubation atmosphere) each plate in the MIC series is inoculated with a set of bacterial strains, adjusted to a standardized cell density. The same set of bacterial strains is replicated onto each plate in the MIC series using a multipoint inoculator. The capacity of the inoculator determines how many strains can be tested simultaneously using a single set of MIC plates.

It is in this stage of the procedure that a well-designed inoculator can significantly increase the efficiency of the agar dilution MIC procedure. For more than 30 years, DWS has used a traditional “reciprocating” multipoint inoculator, which accommodates an inoculum tray (to hold the bacterial suspensions) and one agar plate. Plates are inoculated one at a time and must be removed before placing the next plate. The maximum number of bacterial strains that can be applied to a single plate is 25 and it is necessary to use the inoculator’s own specific inoculum tray, which must be cleaned and sterilized between experiments.

The HMI-60&24 multipoint inoculator (HAUK Technology & Development Co., Ltd) has dramatically increased the efficiency of agar dilution MIC determinations conducted in our laboratory. It has addressed our requirement to test multiple antimicrobial agents against large panels of anaerobic, potentially therapeutic bacterial strains while reducing the number of MIC plate series that must be prepared for each agent.

The efficient design of the HMI-60&24 inoculator head accommodates 60 pins, allowing this number of bacterial strains to be accurately deposited onto a standard 90 mm agar plate. Although this necessitates inoculum sites that are tightly spaced, a special treatment applied by the manufacturer to the steel pins eliminates dripping of the inocula and produces well defined inoculum sites on various agar formulations. In our work we use supplemented



Figure 1, Blood Agar with 60 pins inoculum, Don Whitley Scientific Limited

Brucella Blood Agar (CLSI standard protocol), Fastidious Anaerobe Agar and supplemented Brain Heart Infusion Agar depending on bacterial strain type and customer requirements. The inoculum container for the HAUK instrument is a standard 96 well microtitre plate, which is especially convenient to load and is disposable, reducing maintenance and setup time. The treated stainless steel inoculator heads and pins are easily cleaned and sterilized.

The rotating design of the HMI-60&24 inoculator provides a further improvement in efficiency. The platform accommodates the inoculum tray and two agar plates, which are inoculated sequentially using bidirectional rotation of the platform. Thus, the first plate can be removed and replaced while the second is being inoculated, allowing continuous operation until the complete series of MIC plates has been inoculated without the need to stop the instrument. Two speeds can be selected facilitate this way of working. Alternatively, each pair of plates can be inoculated and the instrument paused (via the touchscreen or foot switch) while the two plates are replaced.

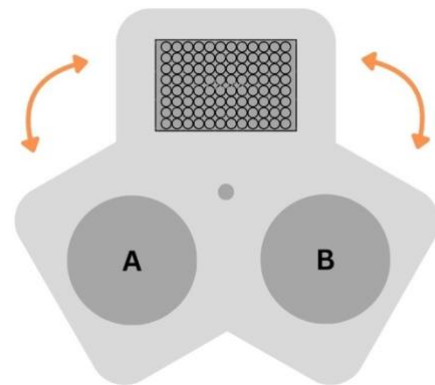


Figure 2

One of the key requirements for our application was an inoculator that could be accommodated inside an anaerobic workstation and operate within its internal atmosphere (temperature 37°C and relative humidity up to 80%). HAUK Technology & Development Co. was able to guarantee that the HMI-60&24 instrument would function reliably under these conditions, and it has done so for several months so far. The shape of the inoculator

makes efficient use of space within the anaerobic workstation because it is relatively tall and deep, while the width is minimized: this leaves the maximum possible working space for other anaerobic work and plate incubation.

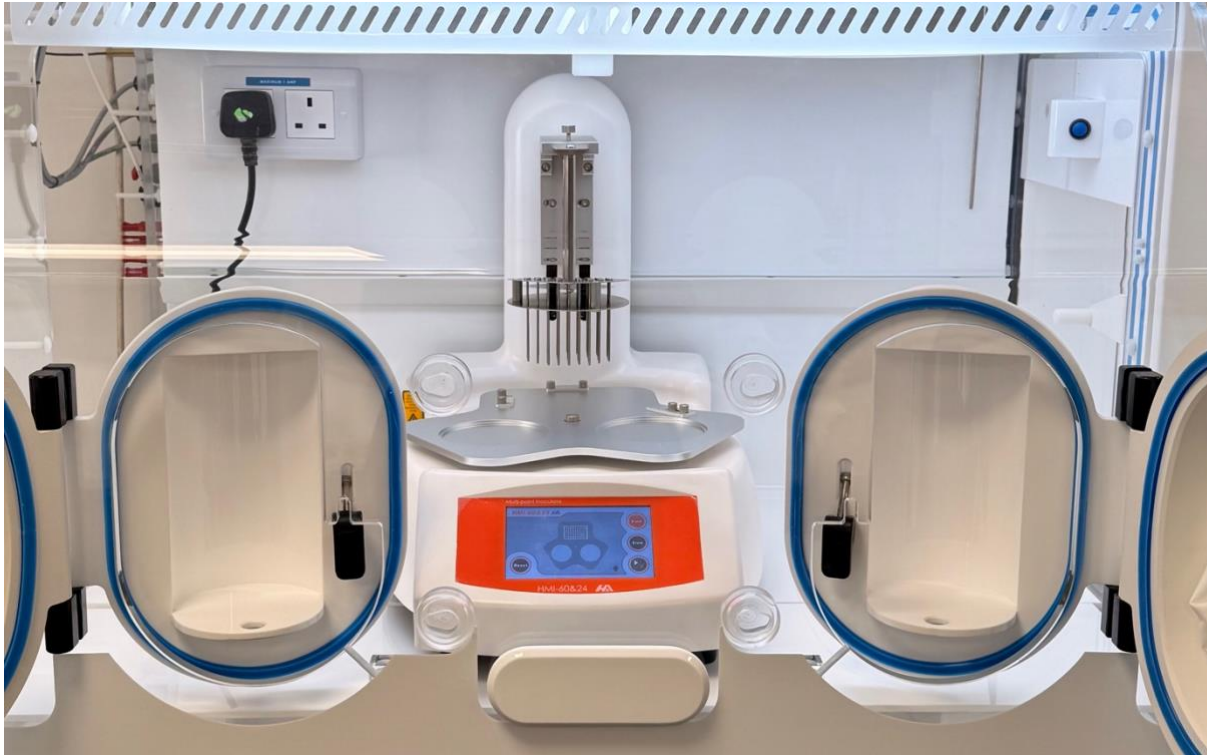


Figure 3, HMI-60&24 Multi-Point Inoculator in Don Whitley Scientific A85 Anaerobic Workstation

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