Rapid antimicrobial susceptibility testing and determination of MIC value using the oCelloScope

Introduction

As a consequence of the dramatic increase in microbial resistance and the recurrent need for treatment with newer and often more expensive antibiotics, there is currently an accentuated focus on the ability to develop cost-effective, fast and accurate antimicrobial susceptibility testing (AST) methods [1,2]. The most widely used AST methods include manual tests such as disk diffusion and broth microdilution[3], as well as phenotypic[4] and genotypic[5] techniques. Manual tests provide flexibility, possible cost saving and quantitative results (e.g., determination of minimum inhibitory concentration, MIC)[6], although they may not accurately predict the results of many clinical samples[7]. Additionally, new emerging mechanisms of bacterial resistance require continuous revision of the adequacy of each AST methods[8].

The oCelloScope is a robust, automated digital time-lapse bright field imaging system, that enables rapid higher throughput, non-invasive, real-time monitoring of microbial growth, growth inhibition and morphological features. The oCelloScope allows the scanning of volumes by recording a series of images to form an image stack where all the microorganisms are caught in focus. The system consists of the small portable oCelloScope instrument, which can fit inside standard laboratory incubators, and the UniExplorer software for instrument control and data analysis. The oCelloScope supports several types of sample containers including microscope slides and microtiter plates up to 96 wells. It is well suited for liquid samples and can be used for accurate and sensitive AST providing short time-to-result. It allows fast screening of up to 96 bacteria-antibiotic combinations in a time as quick as 2 min 19 sec when a single image per well is acquired. The UniExplorer software generates time-lapse videos of the acquired images, growth and growth inhibition curves, as well as performing quantitative analysis of morphological features.

Facts

AST using the oCelloScope showed a statistically significant antibiotic effect within 6 min for *Escherichia coli* monocultures and within 30 min in complex samples from pigs with catheter-associated urinary tract infections[9]. Additional investigations demonstrated the suitability of the system to early detect the resistance profiles of bacteria reference strains and multi-drug-resistant clinical isolates[10]. Furthermore, image analysis performed with the oCelloScope was shown to allow measuring bacterial length and morphological changes, thereby differentiating between normal growth patterns and bacterial filamentation[11]. This would otherwise be impossible using conventional optical density measurements. This is particularly relevant when testing β-lactam antibiotics such as penicillins, cephalosporins, carbapenems and monobactams, which typically induce morphological changes in bacteria such as filamentation and spheroplast formation.

Fredborg et al. showed that the oCelloScope provides a very high accuracy (96% overall agreement) when determining the resistance profiles of four reference strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212 and *Streptococcus pneumoniae* ATCC 49619), nine clinical isolates, including multi-drug-resistant isolates, and three positive blood cultures[10]. AST of clinical isolates (168 antimicrobial agent–organism combinations) demonstrated 3.6% minor, no major and 1.2% very major errors of the oCelloScope compared to conventional susceptibility testing, according to guidelines which state that categorical agreements, major errors and very major errors should be > 90%, < 3 % and < 1.5%, respectively.[12] The oCelloScope also showed rapid and correct phenotypic detection of strains with methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase profiles. The net average time-to-result was 108 min[10].

The oCelloScope can be used for performing AST on reference strains and bacterial isolates following two alternative approaches:

- Preparing the antibiotic dilution range in growth medium using series of doubling dilution (e.g., 16, 8, 4, 2, 1, 0.5 µg/mL) and adding a proper volume to each well containing the bacterial suspension in a transparent flat bottom 96-well plate. The recommended total volume of bacterial suspension and antibiotic solution to use is 50 – 200 µL. Smaller volumes can be used as long as the sample fully covers the bottom of the well during the entire period of analysis.

- Using commercially available pre-treated microtiter plates, according to the manufacturer's instructions. Examples are the round-bottomed Sensititre® well plates (TREK Diagnostic Systems, Cleveland, OH, USA) and the flat-bottomed well plates from MICRONAUT identification systems (MERLIN Diagnostika GmbH, Bornheim, Germany). The oCelloScope however, can only measure on flat-bottomed well plates, therefore if using pre-treated round-bottomed plates, the bacterial samples that have been added to the round-bottomed wells should be promptly transferred to a transparent flat bottom 96-well plate for the oCelloScope analysis.

Note: In both cases, it is always recommended to run the experiment in triplicate including positive controls, where the same volume of plain growth medium is added.

In the present application note, we describe how to perform AST and determine the minimum inhibitory concentration (MIC) value for an antimicrobial using the Sensititre® system and the oCelloScope. Examples of procedures for bacteria cultivation prior to the analysis are also suggested below and can be modified and further optimised depending on the sample source and the bacterial strains.

Protocol for AST and determination of MIC value using the Sensititre® system and the oCelloScope

1. Place the oCelloScope in a standard laboratory incubator for biological applications for precise temperature regulation. Prior to performing the AST experiment, it is recommended to let the instrument and the sample container equilibrate inside the incubator for at least 2 hours. This step allows prevention of condensation forming on the microtiter plate lid, which would otherwise affect the quality of the acquired images and, consequently, the quantitative analysis.
2. Grow reference strains or clinical isolates under atmospheric conditions at 37 °C in CAMHBT (cation-adjusted Mueller–Hinton broth, TREK Diagnostic Systems) overnight. For *S. pneumoniae*, consider using CAMHBT+LHB (lysed horse blood).
3. Transfer bacterial suspensions or isolates to new tubes and incubate for 2 hours before AST analysis.
4. For blood cultures, centrifuge at 200 × g for 5 min to remove human blood cells and resuspend the bacterial pellet in CAMHBT.
5. Dilute the bacterial suspensions to McFarland 0.5 based on OD_{600} measurements.
6. Dilute in CAMHBT to a final concentration of 1×10^5 bacteria/mL. For S. pneumoniae, dilute to a final concentration of 5×10^5 bacteria/mL.
7. Add bacterial suspensions to the Sensititre® 96-well plate and mix thoroughly to ensure fully dissolving of antimicrobials.
8. Carefully transfer bacterial suspension from the round-bottomed Sensititre® plate to a transparent flat bottom 96-well plate, which is compatible with the oCelloScope system for measurements. *Note:* This step is avoided when using MICRONAUT well plates, which are flat-bottomed.
9. Introduce the plate loaded with the bacterial suspensions in the oCelloScope plate holder, tighten firmly and close the instrument lid. Proceed with the AST analysis using the oCelloScope and the Growth Kinetics module.
    - Set the illumination level. Optimal results are typically obtained with a short illumination time (the default value is 2 milliseconds and it is usually the best solution).
    - Find the focus. Among the three focus algorithms for automated focus adjustment, 'Bottom search' is suitable when working with the microtiter plate types included in the UniExplorer list.
    - Use the default scan area.
    - Adjust the time of analysis by selecting the number of acquired images ('Number of repetitions') and the time interval between two sequential images ('Repetition interval'). For instance, by selecting 'Number of repetitions' = 33 and 'Repetition interval' = 00:15:00, the oCelloScope will take the images every 15 minutes for eight hours with the first image taken at t = 0. By ticking 'Use multiple repetition intervals', it is possible to set two different phases of analysis with different time intervals between sequential images. Such intervals can be adjusted according to the expected phases of bacterial growth, depending on the microorganism type. An acquisition time of 12 hours with a 'Repetition interval' of 15 min is suggested for AST analysis, resulting in 49 total repetitions.
10. Use antimicrobial breakpoint tables provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) in Europe and the Clinical & Laboratory Standards Institute (CLSI) in the United States to interpret MIC values and classify the bacterial reference strains and isolates as susceptible, intermediate or resistant.

Fig. 1 shows an example of determination of the MIC value using the oCelloScope and the Growth Kinetics module. The graph reports growth curves for *Staphilococcus aureus* ATCC29213 treated with vancomycin in the concentration range of 0.25 – 32 mg/L. Positive control is included. The MIC value is 1 mg/mL.

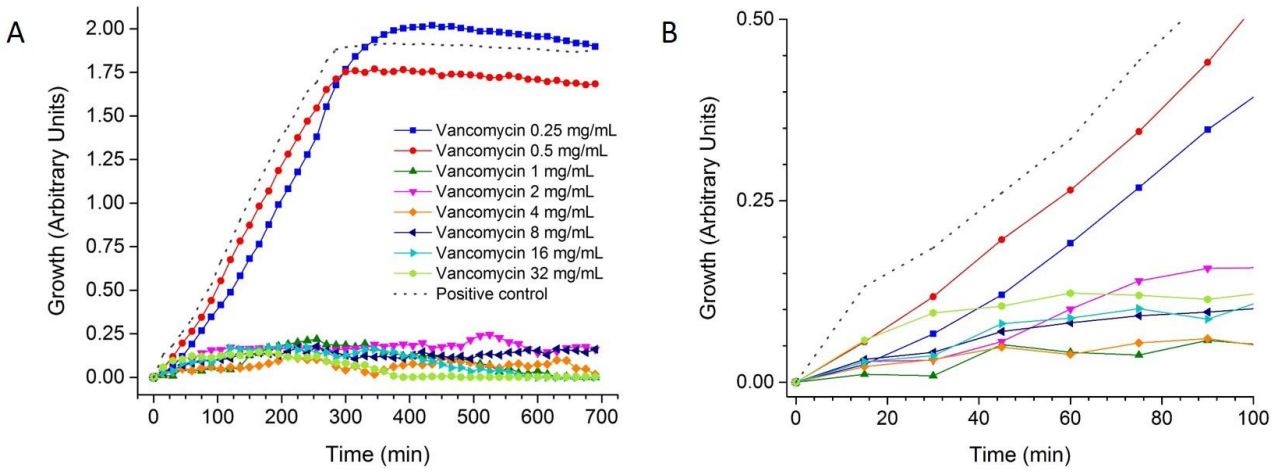

Figure 1. Determination of the MIC value using the oCelloScope and the Growth Kinetics module (A) with focus on the first 100 min (B). Growth curves for *Staphilococcus aureus* ATCC29213 treated with vancomycin (0.25 – 32 mg/L) are reported together with a positive control (non-treated bacterial suspension). A starting concentration of 1×10^5 bacteria/mL was add to a Sensititre® well plate and then carefully transferred to a transparent flat bottom 96-well plate, compatible with the oCelloScope system for measurements. The MIC value is 1 mg/mL.

Additional Notes

- When preparing multiple samples in a microtiter plate, it is recommended to add the same volume to each well. This allows the same settings for illumination and focus to be applied to all wells.
- When analysing samples with few and/or small bacteria (10^3 – 10^4 bacteria/mL and ~ 1 µm, respectively), it may be beneficial to add polystyrene beads of an appropriate size (e.g., 2×10^4 6-µm beads/mL, microsphere standard, B7277, Invitrogen, Nærum, Denmark) to facilitate the focusing process[10]. During the analysis, the segmentation algorithm will be able to ignore the beads.

References

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Warnings and precautions

During sample preparation, biosafety guidelines for handling biological specimens and waste should be followed. For other reagents, refer to the material data sheet from the pertaining manufacturer.

Limitations

The oCelloScope has not been validated for use in diagnostic procedures, including IVD studies. The system is for research use only.

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