Evaluation of a new inoculum standardization device and a fast susceptibility testing medium for *Staphylococcus aureus* antimicrobial susceptibility testing

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SUMMARY

New device *INOCLIC™* for inoculum preparation and standardization, and new rapid medium MHR-SIR™ were tested for *Staphylococcus aureus* A.S.T.

Sixty five different clinical strains of *S. aureus*, including fifteen Meticillin-Resistant *S. aureus* (MRSA) strains, were tested using:
Two different methods for inoculum standardization: *Inoclic* device (i2a, Montpellier, France) versus standard 0.5 Mac Farland suspension according to CLSI guidelines.
And two different culture media: classical Mueller-Hinton medium (MH, i2a, Montpellier, France) versus fast Mueller-Hinton medium (MHR-SIR™, i2a, Montpellier, France).

- Results obtained with the *Inoclic* device were quite identical with those obtained with standard 0.5 McF suspension: concordance between the two methods equaled to 99.51% and correlation ratio equalled to 0.9454.
- With MHR-SIR™ and the *Inoclic* device, after 6h of incubation, we observed 97.57% concordance compared with MH 0.5 McF after 18h of incubation. Moreover, 14/15 MRSA strains were detected within 6h directly by the cefoxitin test, the 15th requested an additional incubation.

MATERIAL AND METHODS

The *Inoclic™* is a device designed to prepare and standardize the inoculum for agar diffusion susceptibility testing directly from a bacterial culture (1, 2). The *Inoclic™* device is a calibrated rod that collects a constant amount of bacteria corresponding to the desired bacterial load inoculum for agar diffusion susceptibility testing. The use of the device is very simple: the operator picks the first colony at right angles to the agar, goes through it and then removes the device by making the reverse movement. Other colonies of the same morphology can be picked from the surface; parts of collected colonies are then suspended in a tube containing 0.7 ml of physiological saline to meet the bacterial load recommended by the CLSI (3) and EUCAST (4) guidelines.

Sixty five strains of different*S. aureus* clinical strains, including 14 MRSA (30% of strains) were used to compare the susceptibility testing made from the *Inoclic™* method to the 0.5 McF standard method.

Bacterial suspensions were inoculated on the culture medium plate using a SirScan swab (i2a, France).

For each suspension prepared with the *Inoclic™* device or adjusted to 0.5 McF, an antibiogram was carried out on an MH susceptibility media (i2a, France), comprising 19 antibiotic discs (SirScan™ disks, i2a, France) including: Penicillin, Cefoxitin, Moxalactam, Kanamycin, Tobramycin, Chloramphenicol, Tetracyclin, Minocyclin, Erythromycin, Quinupristin-Dalfopristin, Linezolid, Ofloxacin, Ciprofloxacin, Levofloxacin, Moxiflofloxacin, Trimethoprime + sulfamethoxazole, Vancomycin, Teicoplanin and Nitrofurantoin. Dropping of antibiotic disks was performed as recommended by the CLSI (M02-A10) (3).

With the suspension prepared with the *Inoclic™* device, 65 AST were also carried out on the MHR-SIR™ medium (i2a, Montpellier, France). MHR-SIR™ is a medium containing a base Mueller-Hinton, enabling antibiogram results within 6 hours (5). All AST cultures were twice read: after 6 and 18 hours of incubation.

The use of the *Inoclic™* device is mandatory to achieve the inoculum for susceptibility testing using MHR-SIR™. Reading MHR-SIR™ media must be done using a SirScan automated reader (i2a, Montpellier, France); the reader utilizes an algorithm for reading the growth of the micro-organism and an expert system analyzes the reading and performs reliability checks if necessary.

The automated reader SirScan2000 Automatic (i2a, Montpellier, France) was used to perform the incubation and the reading of inhibition diameters of all antibiograms performed (Fig. 1)

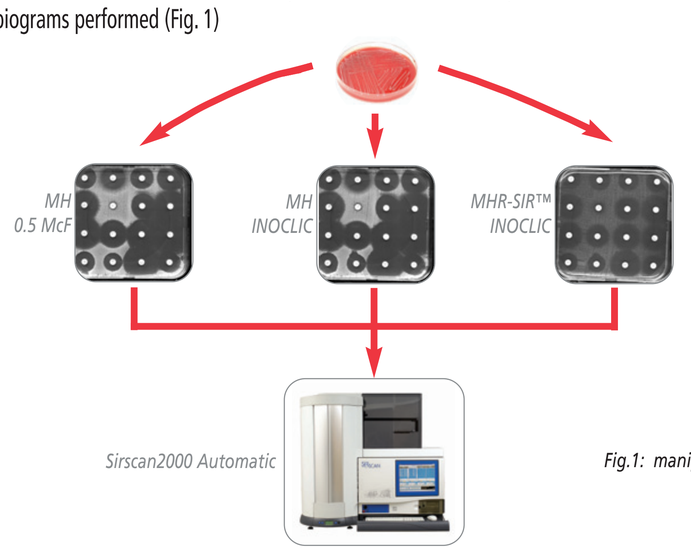

Fig.1: manipulations schema

For diameters data analysis, we defined three types of differences:
- "Minor difference" was used when a molecule was categorized "Sensitive" by a method (or a medium) and "Intermediate" by the other or "Intermediate" by a method (or a medium) and "Resistant" by the other
- "Major difference" was used when a molecule was categorized "Resistant" by the *Inoclic™* device or the MHR-SIR™ medium and "Sensitive" by the standard 0.5 McF suspension or the MH medium.
- "Very major difference" was used when a molecule was categorized "Sensitive" by the *Inoclic™* device or the MHR-SIR™ medium and "Resistant" by the standard 0.5 McF suspension or the MH medium.

All statistical analysis were performed on Excel software.

DISCUSSION

- **The *Inoclic™* device for preparation and standardization of inoculum gives equivalent results to the standard 0.5 McF method for susceptibility testing of *S. aureus* on MH medium. As it is easy and quick of use, the *Inoclic™* device can be used in daily practice of medical laboratories. Results observed in this work confirm preliminary results described (1,2).**

- **In addition, AST using MHR-SIR™ medium shows an excellent correlation with the standard method using classical MH medium. Besides, it has the advantage of giving results after 6h of incubation, even with the majority of resistant strains of MRSA type tested, which could contribute to accelerate the diagnosis and improve the patient treatment.**

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4 - Eucast guidelines available on the net at "eucast.org "
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CONCLUSION

The use of the *INOCLIC™* device for the preparation and standardization of the inoculum for *S. aureus* AST can be recommended for daily practice.
Besides, MHR-SIR™ medium reduces AST time from 18 to 6 hours for *S. aureus*, including MRSA strains.

RESULTS

Comparison of the *Inoclic™* device with the standard 0.5 McF suspension

Inhibition diameters of 19 antibiotic discs for the 65 *S. aureus* strains tested were measured for the two methods.
For the 1234 inhibition diameters obtained, the correlation ratio of the diameters amounted to 0.9454. We observed 0.24% minor differences, no major difference, 0.24% very major errors differences and 99.51% concordance between the *Inoclic™* device and the reference method.
The correlation ratio of the diameters amounted to 0.9454. (Fig.2-3).

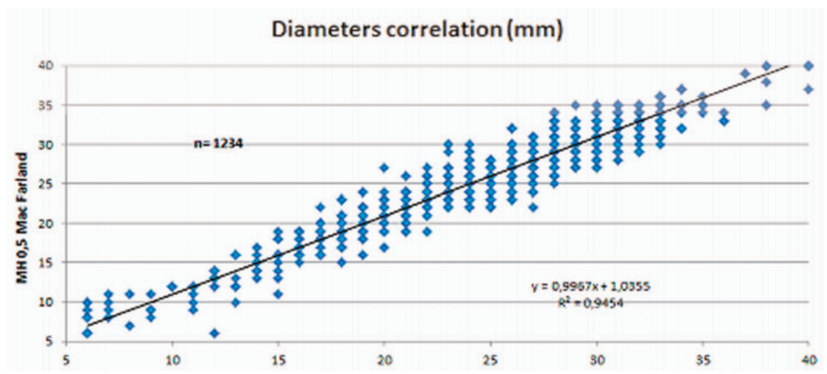

Fig.2: Correlation between the inhibition diameters obtained with the Inoclic™ method (X axis) and the 0.5 McF reference method (Y axis). correlation ratio R^2 equals to 0.9454

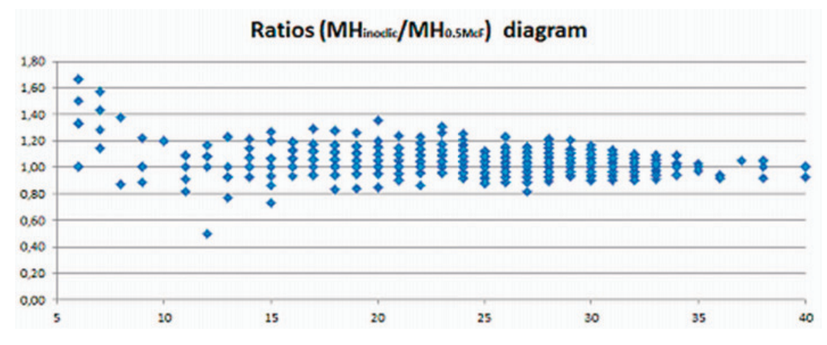

Fig. 3: Diagram discriminating between inhibition diameters obtained with Inoclic™ device and standard 0.5 McFarland method. X axis represents inhibition diameters and Y axis diameters ratio between the two methods. Each blue point corresponds to an Inoclic™/0.5McF ratio.

A majority of ratios was comprised between 1.2 and 0.8, illustrating the good concordance between inoculum performed with the *Inoclic™* device and with the standard 0.5McF method. The worst ratios were observed with the smallest zone diameters what was expected as a difference of only 1 or 2 mm on a small zone diameter led quickly to a ratio > 1.2 or < 0.8.

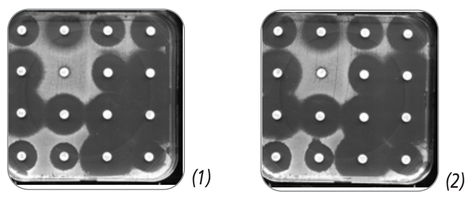

Fig. 4: Visual comparison between 2 AST performed on MH medium with reference method 0.5 McFarland (1) and Inoclic™ device (2). There is no difference between the two plates.

Comparison of the AST obtained with the MH medium and the MHR-SIR™ medium

For AST using rapid media MHR-SIR™ after 6h of incubation, there were 1.94% minor difference, 0.08% major difference, 0.41% very major difference, and 97.57% concordance with the results obtained with classical MH medium (after 18h) inoculated with a 0.5 McF suspension (Fig. 5).
The concordance rose to 99.03% when AST on MHR-SIR™ were read after 18h of incubation.

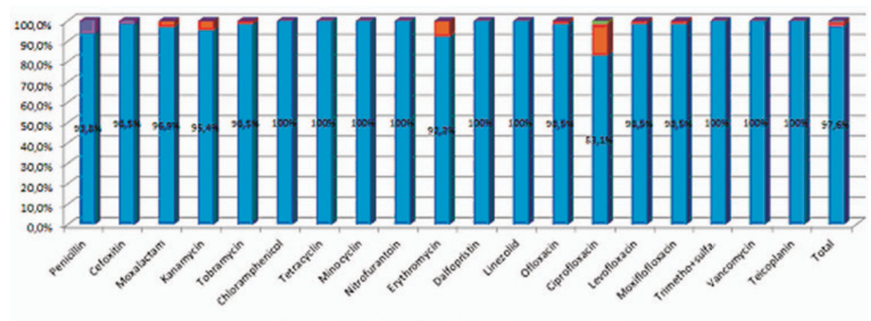

Fig. 5: Results correlation between MHR-SIR™ after 6h of incubation and MH after 18h of incubation. All molecules tested are presented on the X axis and the concordance percentage is figured on the Y axis

After 6h of incubation, among the 1234 inhibition diameters obtained, we observed:

Five very major differences (0.41%) on only 2 molecules: Penicillin (4 cases) and Cefoxitin (1 case).
Penicillin was classified either sensitive or resistant depending on the medium used. However, even if the Penicillin appeared sensitive, it required the cefinase test to confirm the result.

Concerning the Cefoxitin, all Meticillin-Sensitive *S. aureus* (MSSA) strains were detected within 6h of incubation and showed a zone diameter ≥ 22 mm with the cefoxitin disc and a zone diameter ≥ 23 mm with the moxalactam disc. Fourteen out of fifteen MRSA strains were also detected within 6h of incubation. All of these strains had a zone diameter ≤ 21 mm with the cefoxitin disc and a zone diameter ≤ 22 mm with the moxalactam disc. There was a very major difference on the cefoxitin of the last MRSA strain. This strain showed discordance between the zone diameter of the cefoxitin (22 mm, i.e. sensitive) and the zone diameter of the moxalactam (19 mm, i.e. intermediate) after 6h of incubation. After 18h, the strain was correctly categorized MRSA with a zone diameter ≤ 21 mm with the cefoxitin disc (19 mm) and a zone diameter ≤ 22 mm with the moxalactam disc (19 mm).

Twenty-four minor differences (1.94%) on 8 molecules, principally on Erythromycin (5 cases) and Ciprofloxacin (10 cases). Erythromycin was categorized intermediate (with MHR-SIR™) and resistant (with MH), what suggested in both cases not to use this molecule for treatment.

One major difference (0.08%) and ten minor differences were observed on Ciprofloxacin. The molecule was categorized either sensitive or intermerdiate depending on the strain and the AST medium used. Prolonging the incubation to 18h only partially resolved these differences, suggesting testing another fluoroquinolon (ofloxacin, levofloxacin, moxifloxacin) with better concordance.

On the contrary, a perfect concordance with MHR-SIR™ after 6h of incubation and MH after 18h of incubation was observed for 9 molecules such as Tetracyclin, Minocyclin, Nitrofurantoin, Chloramphenicol, Dalfopristin, Linezolid, Vancomycin, Teicoplanin, Trimethoprim+sulfamethoxazole, allowing an adapted treatment faster than with usual AST media.