Multicentric evaluation of the reliability and the reproducibility of synergy testing using the MIC test strip – synergy application system (MTS-SAS™)

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INTRODUCTION

In a time where the antimicrobial resistance is a major challenge for clinicians and clinical microbiologists, the use of synergy testing is sometimes requested.

Among the different methodologies which can be used for this purpose, the checkerboard technique is often considered as the gold standard: it is easy to understand, the procedure and the results analysis are standardized, and can be performed in many clinical microbiology laboratories. The limitations of this method are that it only tests antimicrobials for a fixed incubation time and that it can require a large number of reagents and resources to test different antimicrobial combinations.

The multiple-combination bactericidal test allows for the testing of many antimicrobials simultaneously, but only fixed concentrations are assessed and it requires skilled personnel to be correctly performed.

Time-kill assays provide reliable synergy results at multiple times during the growth phase of bacteria, but are difficult to perform and correctly evaluate.

Gradient diffusion tests have also been proven useful. Two different protocols have been used: nowadays, two strips, each containing one of the antimicrobials of interest, are placed perpendicular to each other, intersecting at the MIC for each antimicrobial when tested alone. Recently a commercial gradient diffusion test (MTS-SAS™, MIC test strip – synergy application system, Liofilchem®, Italy) has been proposed: it uses patented tools designed to facilitate the operators in performing the tests, therefore increasing the reproducibility.

The aim of this study was to evaluate the reliability and reproducibility of the MTS-SAS™ among 12 different Italian primary hospitals. The centers which took part in the study did not receive any specific training to carry out the procedure.

MATERIALS AND METHODS

Strains' selection and drugs tested – Ten bacterial strains were sent to 12 different clinical microbiology Laboratories of the Italian hospitals. The isolates were: 3 *Pseudomonas aeruginosa,* 2 of them multidrug-resistant (MDR) and 1 wild-type; 3 *Acinetobacter baumannii (*2 MDR and 1 wild-type), 3 *Klebsiella pneumoniae* MDR (1 isolate harboring bla_{VIM}, 1 bla_{KPC} and 1 bla_{CTX-M}) and 1 *Escherichia coli* MDR (harboring bla_{KPC}), whose MICs for the antibiotics to be tested were previously determined by the reference laboratory using the broth microdilution technique (BMD).

Single MIC determination for ciprofloxacin, meropenem, colistin, ceftazidime, amikacin, tigecycline and rifampin (the last two drugs, only on *A. baumannii* strains) were tested by the 11 centers against the 10 strains.

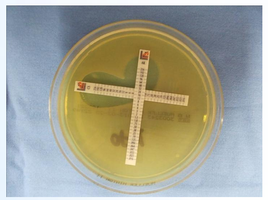
Synergy tests were then performed for the above antibiotics: the antibiotic combinations tested are shown in Table 1.

MTS-SAS™ - The test was performed according to the manufacturer's instructions. Briefly, after having determined the MICs for the single drugs, two MIC test strips™, each containing one of the antimicrobials to be tested, are placed perpendicular to each other in the MTS Synergy Applicator System™ (Figure 1), intersecting at the MIC for each antimicrobial when tested alone. The two strips are then picked up using the MTS Synergy Delivery Tool™ (Figure 1 and 2), moving them on the surface of an Mueller-Hinton agar plate inoculated with a suspension of the strain to be tested. The strips are then pushed onto the agar surface by using tweezers, in order to facilitate their removal of the MTS Synergy Delivery Tool™.

Results have been stratified in accordance with the FIC index: synergic if <=0,5, antagonist if >4.0 and otherwise indifferent (i.e., not considering the additive definition).

Checkerboard – Finally, two laboratories (Reggio Emilia and Bologna) performed the checkerboard technique on the same drug combinations, to evaluate the overall agreement between the two methods.

Figure 1, left – MTS Synergy Applicator System™ and MTS Synergy Delivery Tool™
Figure 2, right – MTS Synergy Delivery Tool™ : the strips are moved onto the agar surface



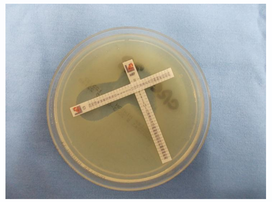


Figure 3, left – Synergy testing with **MTS-SAS™: example of indifference**
Figure 4, right – Synergy testing with **MTS-SAS™: example of synergic effect**

RESULTS

Centers participation – One hospital did not complete the study and their results have been discarded from the final analysis.

Single MICs determination – All the centers tested 5 drugs on the 3 *P. aeruginosa,* 3 *K. pneumoniae* and 1 *E. coli* and 7 drugs on the 3 *A. baumannii* isolates. Thus overall 56 drugs were tested by the 11 centers on the 10 strains (616 different determinations). All the centers, for all the strains, overestimated the MIC of colistin if compared with BMD. Even considering that bias, concordance with BMD (within +/- doubling dilution) was 98.4%. Only in 10 cases, MICs values did not fall into a 3 doubling dilution range. Two out of the 10 cases concerned meropenem determination in 1 *Klebsiella pneumoniae* harboring bla_{KPC}, which showed a phenotypic aspect of heteroresistance.

Synergy testing - Ninety three drug combinations have been analyzed (1023 determinations). Example pictures are shown in Figure 3 and 4. There were 27 synergy tests performed with drugs which have shown MICs not in agreement with BMD (as above) and these were excluded from the final analysis, which was then performed on 996 drug combinations.

MTS-SAS™ interlaboratory agreement: results are shown in Table 1. Discordant results were related only to synergistic effect, instead of indifference and were documented only in 33 cases (3.3%). The combination with the lowest concordance levels were amikacin + ceftazidime (89.8%), followed by amikacin + meropenem (92,3%). It is noteworthy that 19 cases concerned a single strain, the *P. aeruginosa* wild-type isolate.

MTS-SAS™ agreement with checkerboard - The checkerboard technique showed a 95.3% overall agreement (concordance level) with the MTS-SAS™ (Table 1). The combination amikacin-meropenem demonstrated the highest discordance level between the two methods.

Antibiotic combinations	Total tests	MTS-SAS™ agreement among sites (nr - %)	MTS-SAS™ agreement to checkerboard (%)
colistin + meropenem	105	104 (99.0)	99.2
meropenem + ciprofloxacin	105	100 (95,2)	95
amikacin + ceftazidime	108	97 (89.8)	92
colistin + ceftazidime	109	108 (99.1)	99.2
colistin + ciprofloxacin	109	109 (100)	100
amikacin + meropenem	104	96 (92.3)	75.9
amikacin + ciprofloxacin	108	108 (100)	100
ceftazidime + ciprofloxacin	108	102 (94.4)	90
colistin + amikacin	109	108 (99.1)	99.2
tigecycline + rifampin (*)	31	31 (100)	100
TOTAL	996	963 (96.7)	95.3

Table 1 – Antibiotics combinations tested and sinergy results summary. (*) combination used only for testing *A. baumannii* strains. Right columns: MTS-SAS™ interlaboratory agreement and concordance with checkerboard technique.

DISCUSSION

- The evaluation of synergy testing by using the gradient diffusion was performed in the past with a cumbersome technique, which involved placing the two strips on the agar inoculated with a lawn of the test organism, allowing the first strip (containing drug number 1) to act for 60 min and then after its removal, placing a second strip on the agar in the same position. This approach was *de facto* abandoned by a vast majority of laboratories, due to the technical difficulties which led to a poor reproducibility.
- The second approach (i.e. placing the two strips perpendicularly to each other, intersecting at the MIC for each antimicrobial tested alone) was more user-friendly. Recently, the MTS-SAS™ system has been proposed, with the aim of making this methodology more reproducible.
- Our findings indicate that MTS-SAS™ showed a very good interlaboratory reproducibility, with a concordance of results among the different laboratories of 96,7%. The test was determined to be easy to use (no training was given before starting the study).
- Moreover, an overall comparability of MTS-SAS™ and checkerboard assays was observed, suggesting excellent quantitative and qualitative agreement.
- The major discordances (synergistic effect, instead of indifference) were documented in testing a *P. aeruginosa* wild-type isolate (19.2%). This can be alarming, demonstrating indirectly the well-known limitation of synergy techniques. On the other hand, synergy tests are most applicable to MDR strains, which were largely represented in the present study, with excellent agreement.

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