SUMMARY

Background: The method of serum bactericidal assay represents an alternative possibility of optimization of anti-infectious therapy and administration of antibiotics. It mirrors the real activity of one or more administered antibiotics in the complex system of the antibacterial effect of patient´s serum. The paper aimed to confirm non-inferiority of bactericidal testing using the broth dilution method according to CLSI M21-A Guidelines (time to results 48, 72 hours) in comparison with modified methods of testing on the basis of turbidimetry (time to result 6, 8, 24 hours) and resazurin color (time to results 8, 24 hours).

Methods: Four antibiotics were tested: gentamicin, amikacin, piperacillin/tazobactam and meropenem with 30 *Escherichia coli* strains isolated from blood cultures of 29 pacients hospitalised in different wards, University Hospital in Hradec Kralove. Human blood sera (n = 76) from ten hematological patients (4th Department of Clinical Medicine, University Hospital, Hradec Kralove) were tested to establish bactericidal titer. Patients' blood was withdrawn prior to and in the course of the first and third day of antibiotic therapy of febrile neutropenia. Testing employed the reference strain *Escherichia coli* ATCC 25922.

Results: A comparison with the standard CSLI showed that the results of bactericidal testing of antibiotics did not differ at the statistically significant level (non-inferior) in 24-hour modification of the methods used. All results of the modification tests before 24 hours were statistically different from CLSI method. The results of the modified turbidimetric method were non-inferior with the use of the wavelength of 405 nm; the best two-tailed *p*-value was achieved at the break-point < 30% of the change of turbidance. Comparison of the results of serum bactericidal activity showed no dependence on the wavelength used (405 nm versus 620 nm) and provided comparable two-tailed *p*-value after 24-hour incubation.

The modified method using resazurin color was also statistically non-inferior from CSLI provided that color was read after 8-hour incubation and added subculture of contents of negative wells. So the results were available after 24 hours.

In the case of reading after 6 and 8 hours of incubation, the results of both proposed serum bactericidal testing methods exerted statistically significant differences from the standard CLSI.

Conclusion: The proposed modifications, which use turbidimetry and resazurin color for testing of bactericidal activity of serum after 24 hours of incubation yielded results comparable with the CLSI method and thus shortened the time necessary for their achievement by 24 to 48 hours in comparison with the standard methodology according to CLSI.

Moreover, a careful laboratory work keeping all the principles of good laboratory practice is also necessary to obtain valid results. The same is true for correct timing of patient serum collection, flawless collection, storage and transport. Close cooperation of the laboratory workers with clinicians is also necessary as well.

However, the value of serum bactericidal titer, which reliably predicts the clinical effect of patient treatment, remains an issue under discussion. It seems that the conclusions of earlier papers about general recommendations for sufficient serum bactericidal titer will need to be reconsidered and individualized using pharmacokinetic and pharmacodynamic principles. Further laboratory and clinical studies will be necessary to confirm these assumptions.