Rocz Panstw Zakl Hig 2017;68(4):389-394

http://wydawnictwa.pzh.gov.pl/roczniki_pzh/

ORIGINAL ARTICLE

NEUTRALIZATION EFFICIENCY OF ALCOHOL BASED PRODUCTS USED FOR RAPID HAND DISINFECTION

Agnieszka Chojecka[1], Patryk Tarka[2], Anna Kierzkowska[1], Aneta Nitsch-Osuch[2], Krzysztof Kanecki[2]*

[1]National Institute of Public Health – National Institute of Hygiene,
Department of Bacteriology, Warsaw, Poland
[2]Medical University of Warsaw, Department of Social Medicine and Public Health, Warsaw, Poland

ABSTRACT

Background. Alcohols are the most commonly used active substances in preparations for quick hand disinfection. They should be bactericidal in very short contact time. PN-EN 13727 + A2: 2015-12 standard, for testing hygienic and surgical handrub disinfection preparations, provides mandatory test conditions of disinfectants in contact times with the range of 30 s to 60 s (hygienic handrub disinfection) and 60 s to 5 min (surgical handrub disinfection). A short contact times for hand hygiene products require a short time of neutralization process. For contact times less than or equal to 10 minutes, the estimated neutralization time is 10 s ± 1 s. Neutralization is a process that abolishes the action of disinfectants. Correct application of this process allows for proper use of disinfectants in practice and its biocidal effect.

Objectives. Verification of the effectiveness of 10-second neutralization time of alcohol based preparations for hygienic handrub disinfection.

Materials and Method. Neutralization of two products with different ethanol content (89% and 70%) for hygienic handrub disinfection according to PN-EN 13727 + A2: 2015-12 was investigated. The effectiveness of the neutralizer was assessed by determining toxicity of neutralizer, activity of residual effects of the tested products and their derivatives produced during neutralization (10 s) for test organisms (*Staphylococcus aureus* ATCC 6538; *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541; *Escherichia coli* K12 NCTC 10538).

Results. The 10-second neutralization time was sufficient to eliminate the residual activity of products for hygienic handrub disinfection with differentiated ethanol concentration. The neutralizer used did not show toxicity to bacteria and did not produce toxic products with tested preparations after neutralization.

Conclusions. The use of 10-second neutralization time allows in a precise way designate the contact times for hygienic handrub disinfection products.

Key words: *neutralization, hygienic handrub disinfection, ethanol, bactericidal activity*

STRESZCZENIE

Wprowadzenie. Alkohole są substancjami aktywnymi najczęściej stosowanymi w preparatach do szybkiej dezynfekcji rąk. Powinny one działać dezynfekcyjnie w bardzo krótkich czasach kontaktu. Norma PN-EN 13727+A2: 2015-12 przeznaczona do badania preparatów do higienicznej i chirurgicznej dezynfekcji rąk przewiduje obligatoryjne warunki badania preparatów w czasach kontaktu w zakresie od 30 s do 60 s (higieniczna dezynfekcja rąk) i od 60 s do 5 minut (chirurgiczna dezynfekcja rąk). Krótkie czasy kontaktu produktów przeznaczonych do higieny rąk wymagają zastosowania krótkiego procesu neutralizacji. Dla czasów kontaktu poniżej lub równych 10 minut przewidziany czas neutralizacji wynosi 10 s ± 1s. Neutralizacja jest procesem znoszącym działanie preparatów dezynfekcyjnych. Prawidłowe wykonanie tego procesu warunkuje prawidłowe zastosowanie preparatu w praktyce i jego działanie biobójcze.

Cel. Sprawdzenie skuteczności 10-sekundowego czasu neutralizacji wobec preparatów na bazie alkoholu przeznaczonych do higienicznej dezynfekcji rąk.

Materiały i metody. Badano etap neutralizacji dwóch produktów o różnej zawartości etanolu (89% i 70%) przeznaczonych do higienicznej dezynfekcji rąk wg normy PN-EN 13727+A2: 2015-12. Skuteczność neutralizatora oceniano poprzez określenie braku wpływu toksyczności neutralizatora oraz działania resztkowego badanych produktów lub ich pochodnych powstałych w czasie neutralizacji (10 s ± 1s) na badane organizmy testowe (*Staphylococcus aureus* ATCC 6538; *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541; *Escherichia coli* K12 NCTC 10538).

[*] **Corresponding author:** Agnieszka Chojecka, National Institute of Public Health – National Institute of Hygiene, Department of Bacteriology, 24 Chocimska street, 00-791 Warsaw, Poland tel. +48 22 54 21 330, e-mail: achojecka@pzh.gov.pl

Wyniki. 10-sekundowy czas neutralizacji był wystarczający do zniesienia aktywności produktów o zróżnicowanej zawartości etanolu przeznaczonych do higienicznej dezynfekcji rąk. Zastosowany neutralizator nie wykazywał działania toksycznego wobec bakterii oraz nie tworzył toksycznych produktów z badanymi preparatami po neutralizacji.
Wnioski. Zastosowanie 10-sekundowego czasu neutralizacji pozwala w precyzyjny sposób wyznaczyć czasy działania produktów przeznaczonych do higienicznej dezynfekcji rąk.

Słowa kluczowe: *neutralizacja, higieniczna dezynfekcja rąk, etanol, bakteriobójczość*

INTRODUCTION

Hand hygiene is an important factor for preventing the spread of nosocomial infections [2, 4, 10, 12,]. Transfer of microorganisms through the hands of health care workers and patients within the hospital environment is associated with a high risk of cross-contamination of this area [2]. Transmission of infections may not only promote improperly disinfected hands of hospital staff or patients, but also improperly determined parameters of use of disinfectants.

Hygienic and surgical handrub disinfection preparations usually contain active substances such as alcohols, or combinations thereof with iodophores or chlorhexidine [2, 5, 14]. These substances are characterized by short contact times and extensive biocidal spectrum, which allows for short-term disinfection of hands and the fight against clinically relevant pathogens [12]. The manufacturer's declared contact times for alcohol-based preparations for hand disinfection are up to 15 seconds [4, 5, 12]. Testing such parameters when determining the biocidal efficacy of a product requires a suitable test method. Biocidal efficacy of preparations for hygienic and/ or surgical hand disinfection should be determined in accordance with the applicable test methods described in the European Standards or testing methods approved by the relevant national organizations or institutions [8, 14]. The testing program for preparations for hygienic and surgical hand disinfection in the medical field is described in EN 14885 [8]. Parameters for use of these formulations should be determined in two successive stages of phase 2 i.e. phase 2, step 1 (PN-EN 13727 - bactericidal activity, PN-EN 13624 - yeasticidal activity) and phase 2, step 2 (PN-EN 1500 - hygienic handrub disinfection; PN-EN 12791 - surgical handrub disinfection). The dilution-neutralization method described in those standards is used to test the biocidal efficacy of disinfection products [7, 8, 12]. In this method, the biocidal activity of the product at the specified concentration is abolished at the appropriate time of contact by the use of a neutralizer. The neutralizer is usually the mixture of organic and inorganic compounds selected for the active substance present in the test product, neutralizing the biocidal effect of this active substance. An important feature of a neutralizer is the inactivation in time of residual activity of the product or active substance after its specific contact with the test microorganisms in the test [8, 13]. Neutralizer should also not create toxic products after reaction with the active substances contained in the products nor inactivate or inhibit the test organisms used in the test [11]. These features of neutralizer are determined during testing of the disinfectant in the control method of dilution-neutralization (validation C) and in the control of the absence of toxicity of the neutralizer to the test microorganisms (validation B) [3, 7]. Biocidal efficacy tests for hand disinfection should also exclude the effects of other conditions on the dilution-neutralization method on test microorganisms. The required reduction of test microorganisms should be based exclusively on the interaction between the test microorganisms and the test product. Standard PN-EN 13727 + A2: 2015-12 for testing of hygienic and surgical handrub disinfection preparations provides for mandatory test conditions for preparations including contact time in the range of 30 s to 60 s (hygienic handrub disinfection) and 60 s to 5 minutes (surgical handrub disinfection). Short contact times for hand hygiene products require a short neutralization process. For contact times of 10 minute or shorter, the neutralization time is only 10 s [7]. The use of short neutralization times requires confirmation of the effectiveness of this process in relation to the tested products and test microorganisms.

The aim of the study was to investigate the effectiveness of 10-second neutralization time on alcohol based formulas using standard PN-EN 13727 + A2: 2015-12.

MATERIALS AND METHODS

Two products intended to hygienic handrub disinfection was used in the experiment. Products contain ethanol in concentrations: 89 g ethanol in 100 g of product (Et89) and 70 g ethanol in 100 g of product (Et70). A neutralizer with the following composition was used to remove alcohol activity: Polysorbate 80 – 30 g/l; saponin 30 g/l; lecithin 3 g/l in diluent. Products testing were carried out in accordance with PN-EN 13727+A2: 2015-12. For testing the Et89 *Staphylococcus aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442 were selected. *Enterococcus hirae* ATCC 10541 and *Escherichia coli* K12 NCTC 10538 were selected for the Et70. A test suspension of bacteria (N) with a density in the range of

1.5×10^8 cfu/ml to 5×10^8 cfu/ml was prepared for products testing. Tests of products activity were conducted at contact times of 15 s± 5 s (Et89) and 1 min± 5 s (Et70) at temperature 20°C in clean conditions (0,3 g/l bovine albumin solution).

Neutralization time of tested products was connected with the length of their contact times and amounted 10 s ± 1 s. To test the effect of experimental conditions (A), the neutralizer control (B) and the dilution-neutralization method (C), validation suspensions with a density ranging from $3{,}0\times10^2$ cfu/ml do $1{,}6\times10^3$ cfu/ml (validation suspension Nv0) and from $3{,}0\times10^4$ cfu/ml do $1{,}6\times10^5$ cfu/ml (validation suspension NvB) were used. The average density range of the validation suspension Nv0 should be equal to or greater than 30 and less than or equal to 160. Lethal effect of test conditions on bacteria was checked by the procedure of experimental conditions control A. According to the European Standards ready-to-use products are diluted with sterile distilled water. Experimental conditions control A consists in leaving the microorganisms in contact with the organic load and sterile distilled water at the contact time specified in the test corresponding to the contact time provided for the test product. The number of microorganisms (cfu/ml) after the contact time should be greater or equal to half of the average number of microorganisms in the validation suspension Nv0. Verification of the absence of toxicity of the neutralizer on tested microorganisms was done according to neutralizer control B. The neutralizer is left in contact with the NvB validation suspension for the time corresponding to the neutralization time. The number of microorganisms (cfu/ml) after the contact time should be greater or equal to half of the average number of microorganisms in the validation suspension Nv0.

The neutralization efficiency of the examined products was determined by validation of dilution-neutralization method (method validation C). For the validation of the method the products were used in ready-to-use concentrations. Products were exposed for 15 s ± 5 s (Et89) and 1 min ± 5 s (Et70) with organic loading and diluent and then transferred to the neutralizer under test. After 10 s ± 1 s of neutralization time, suspensions of test organisms were added and allowed to stand for 30 minutes. The number of microorganisms (cfu/ml) after 30 minutes time should be greater or equal to half of the average number of microorganisms in the validation suspension Nv0. The studies presented in this publication have been performed under reproducibility conditions as part of microbiological quality control of the studies of bactericidal efficiency of disinfectants and antiseptic. The data is presented as mean and standard deviation of the two independent repetitions [7].

RESULTS

The impact of the selected experimental conditions on the tested bacteria

There was no impact of experimental conditions on the microorganisms tested for either 15 s or 1 min. The absence of any lethal effect in the test conditions was confirmed. Control of experimental conditions in each case met the requirements of PN-EN 13727 + A2: 2015-12 (Table 1).

The effect of the neutralizer toxicity on the tested bacteria

Neutralizer toxicity was assessed against all the bacteria required in PN-EN 13727+A2: 2015-12. To study was used validation suspensions NvB of tested bacteria which density was for *S. auerus* 1.43×10^5 cfu/ ml; for *P. aeruginosa* 8.2×10^4 cfu/ ml; for *E. hirae* 8.9×10^4 cfu/ ml and for *E. coli* K12 1.13×10^5 cfu/ ml. The obtained results, according to the standard, were referenced to the validation suspension Nv0. The number of colonies obtained after the neutralization time was greater than half of the average number of microorganisms in the validation suspension Nv0 (Table 2). The neutralizer used was not toxic to tested microorganisms in neutralization time 10 s ± 1 s.

Validation of the dilution-neutralization method

The neutralizer eliminated residual effects of the tested products (Et89 and Et70) after neutralization within 10 s ± 1 s so that it did not limit the growth of the tested microorganisms. The neutralizer potential was sufficient to withstand the residual effects of products which content varying levels of active substance. The effects of the products resulting from interaction of the neutralizer and the disinfectant on bacteria used in the study were also not observed (Tables 3 and 4).

The activity of products intended for hygienic handrub disinfection in relation to selected test bacteria

Products for hygienic handrub disinfection were active at contact times of 15 s ± 5 s and 1 min ± 5 s for selected test microorganisms. The product containing ethanol in concentration 89% was active against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, while the product contain ethanol at concentration 70% was active against *Enterococcus hirae* and *Escherichia coli* K12. The products achieved the required reduction of 5 lg or higher, and all experimental assumptions for tested products and test organisms and the neutralizer were met (Tables 5 and 6). However, in order to give a positive opinion on the disinfection performance of the products described in the publication, the test conditions must be checked for all 4 test organisms provided for in EN-PN 13727: 2012 + A2: 2015 for each test product separately.

Table 1. Influence of experimental conditions on tested microorganisms according to PN-EN 13727 + A2: 2015-12

Test bacteria	Validation suspension (Nv0)	Experimental conditions control (A)	Product	Contact time
Staphylococcus aureus	128 (±21) cfu/ml	143 (±12) cfu/ml	Et89	15 s± 5 s
Pseudomonas aeruginosa	94 (±30) cfu/ml	77 (±22) cfu/ml	Et89	15 s± 5 s
Enterococcus hirae	80 (±52) cfu/ml	82 (±47) cfu/ml	Et70	1 min± 5 s
Escherichia coli K12	98 (±25) cfu/ml	115(±4) cfu/ml	Et70	1 min± 5 s

Table 2. The effect of neutralizer toxicity on the microorganisms tested in 10-second interactions time with an neutralizer according to PN-EN 13727 + A2: 2015-12

Test bacteria	Validation suspension (Nv0)	Neutralizer control (B)	Product	Neutralization time
Staphylococcus aureus	128 (±21) cfu/ml	141(±17) cfu/ml	Et89	10 s± 1 s
Pseudomonas aeruginosa	94 (±30) cfu/ml	81 (±20) cfu/ml	Et89	10 s± 1 s
Enterococcus hirae	80 (±52) cfu/ml	74 (±51) cfu/ml	Et70	10 s± 1 s
Escherichia coli K12	98 (±25) cfu/ml	101 (±0) cfu/ml	Et70	10 s± 1 s

Table 3. Validation of the dilution-neutralization method according to PN-EN 13727+ A2: 2015-12 for two selected test organisms *S. aureus* and *P. aeruginosa* using a product containing ethanol in concentration 89% (Et89)

Test bacteria	Validation suspension (Nv0)	Method validation (C)	Product	Contact time/ Neutralization time
Staphylococcus aureus	128 (±21) cfu/ml	68 (±6) cfu/ml	Et89	15 s± 5 s/ 10 s± 1 s
Pseudomonas aeruginosa	94 (±30) cfu/ml	92 (±28) cfu/ml	Et89	15 s± 5 s/ 10 s± 1 s

Table 4. Validation of the dilution-neutralization method according to PN-EN 13727 + A2: 2015-12 for two selected test organisms *E. hirae* i *E. coli K12* using a product containing ethanol in concentration 70 % (Et70)

Test bacteria	Validation suspension (Nv0)	Method validation (C)	Product	Contact time/ Neutralization time
Enterococcus hirae	80 (±52) cfu/ml	79 (±53) cfu/ml	Et70	1 min± 5 s/ 10 s± 1 s
Escherichia coli K12	98 (±25) cfu/ml	101 (±8) cfu/ml	Et70	1 min± 5 s/ 10 s± 1 s

Table 5. Average reduction of *Staphylococcus aureus* and *Pseudomonas aeruginosa* microorganisms tested according to PN-EN 13727+A2: 2015-12 after 15 seconds contact time with a product containing ethanol at concentration 89% (Et89)

Test bacteria	Test suspension (lgN0)	Reduction [lgR]	Product	Contact time
Staphylococcus aureus	7,56 (±0,01)	>5,41(±0,01)	Et89	15 s± 5 s
Pseudomonas aeruginosa	7,53 (±0,01)	>5,38(±0,01)	Et89	15 s± 5 s

Table 6. Average reduction of tested *Enterococcus hirae* and *Escherichia coli* K12 microorganisms according to PN-EN 13727 + A2: 2015-12 after 1-munute contact time with a product containing ethanol in concentration 70% (Et70)

Test bacteria	Test suspension (lgN0)	Reduction [lgR]	Product	Contact time
Enterococcus hirae	7,54 (±0,03)	>5,39 (±0,03)	Et70	1 min± 5 s
Escherichia coli K12	7,55 (±0,01)	>5,40 (±0,00)	Et70	1 min± 5 s

DISCUSSION

Correctly applied neutralization allows for precise determination of time of action of the product, in which microorganisms will be combat [11, 13]. This is especially important when short-term disinfection agents are used, including those intended for hygienic handrub disinfection. The neutralizer is selected for the active ingredient of the disinfectant. PN-EN 13727+A2: 2015-12 standard provides information of the composition of neutralizers selected for active substances (dilution-neutralization method) or rinsing liquids (membrane filtration method). The filtration method is an alternative when the proposed neutralizers do not eliminate effects of the product action at a certain time of neutralization. The neutralization time for products, which acting at 10 minutes or less was limited to 10 s ± 1 s [7]. To accurately determine contact time of the disinfectant preparation and its bactericidal activity, the effectiveness of the selected neutralizer should be checked at the required neutralization time. Neutralization efficiency according to Russell refers to following features of a neutralizer. The neutralizer should not be toxic to the tested microorganisms as it provides a bactericidal effect that will not result from the action of the disinfectant itself. The neutralizer should inhibit the residual effect of the disinfectant product after the specified contact time so as to prevent further action of the product and the neutralizer and the product must not combine to produce a toxic components. Our results confirmed the effectiveness of the applied neutralizer. The tested neutralizer composed with Polysorbate 80, saponin, lecithin was not toxic to *S. aureus*, *P. aeruginosa*, *E. hirae* and *E. coli* K12. Similar results with multicomponent neutralizers containing, inter alia, such neutralizing substances as Polisorbate 80 and lecithin were obtained by Sutton et al. for such bacteria as *E. coli*, *P. aeruginosa*; *S. aureus* and *S. choleraesuis*. The most sensitive organism for the neutralization of these bacteria was *E. coli*. Two of the four tested neutralizers which contain Polysorbate 80, Polysorbate 20, lecithin and dextrose were toxic for this bacteria, with the concentration of both polysorbats being much higher than in non-toxic neutralizers [11]. The qualitative and quantitative composition of neutralizers determines the toxicity or it absence against the microorganisms and the effectiveness of neutralization of disinfectants. The qualitative and quantitative composition of the neutralizer under test has allowed to inhibit the residual activity of products with vary content of ethanol (Et70 and Et 89 product) intended to hygienic handrub disinfection in the 10-second neutralization time. Proper recovery of *S. aureus* and *P. aeruginosa* microorganisms after neutralization of the product containing ethanol in concentration 89% and *E. hirae* and *E. coli* K12 after neutralization of the product containing ethanol in concentration 70% was demonstrated. Effectiveness of the 10-second neutralization time on the alcohol based products for skin disinfection with *S. auresus*, *P. aeruginosa*; *E coli* and *E. hirae* have confirmed in their study *Tyski* et al. with the use of a universal, commercial Dey/Engley neutralizer containing more chemical components [13].

The concentration of the residual products after disinfection may depend on concentration of disinfectant, its contact time, susceptibility to organic contamination and density of suspensions of the tested microorganisms. These factors minimize the amount of active residual products remaining after the reaction of the disinfectant product with the microorganisms and can contribute to increasing the effectiveness of the neutralization. Products containing high concentrations of active substances and active substances with high disinfection potential can be difficult to inactivate [11]. Substances with high disinfection potential, which are difficult to be neutralized, especially in high concentrations, include such oxidizing compounds like chlorine, iodine, hydrogen peroxide, peracetic acid, hypochlorites [3]. For these substances, new neutralizers are sought [3]. In such cases, the ingredients of neutralizers are modify eg. by increasing the concentration of sodium thiosulphate in the range from 3 g/l to 20 g/l, because in higher concentrations it is toxic to the tested organisms. Addition of an neutralizer to the culture medium is used, or neutralization with the use of rinsing liqiuds in the filtration method [7]. Obtaining neutralization respectively to contact time of the investigated product allows for precise determination of its action over time and at the same time contributes to the effective application of disinfectants in practice, including products with short time, which are intended for hygienic handrub disinfection.

CONCLUSIONS

1. The 10-second neutralization time is sufficient to remove the residual activity of the hygienic handrub disinfectants which content ethanol at concentrations of 70% to 89% using a neutralizer composed of Polysorbate 80-30 g/l ; saponin 30 g/l; lecithin 3 g/l in diluent.
2. Correct neutralization makes it possible to accurately determine the contact times of disinfectants.

Acknowledgements
This research was financially supported by the National Institute of Public Health-National Institute of Hygiene, Warsaw, Poland.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

1. *Bocian E., Tyski S.:* Alcohols application in antisepsis. Zakażenia 2003; 3: 68-74 (in Polish).
2. *Czechowska D., Fleischer M., Lechowicz M., Malara M., Ochocka B., Ziółkowski G.:* Hygiene of hands in health care institutions. Stowarzyszenie Pielęgniarek Epidemiologicznych. 2014; Zeszyt XIII: 5-13 (in Polish).
3. *Eissa M.E., Nouby A.S.:* Recovery of Gram-Positive Cocci and *Candida albicans* from Peroxygens/Silver-Based Disinfectants. Pol J Microbiol 2016; 65: 13-21.
4. *Kampf G., Hollingsworth A.:* Comprehensive bactericidal activity of an ethanol-based gel in 15 seconds. Annals of Clinical Microbiology and Antimicrobials 2008;7:1-6.
5. *Kampf G., Kramer A.:* Epidemiologic Background of Hand Hygiene and Evaluation of the Most Important Agents for Scrubs and Rubs. Clin Microbiol Rev 2004;17:863-893.
6. *McDonnell G., Russell D.:* Antiseptics and Disinfectants: Activity, Action and Resistance. Clin Microbiol Rev 1999;12:147-179.
7. *PN-EN 13727+A2: 2015-12*. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bacteria activity in the medical area. Test method and requirements (phase 2, step 1).
8. *PN-EN 14885: 2015-10.* Chemical disinfectants and antiseptics. Application of European Standards for chemical disinfectants and antiseptics.
9. *Russell AD.:* Neutralization procedures in the evaluation of bactericidal activity. In: Collins C,H., Allwood M.C., Bloomfield S.F., Fox A.eds. Disinfectants: Their Use and Evaluation of Effectiveness. London, Academic Press, Inc. 1981; pp 45-59.
10. *Sickbert-Bennett E. E., DiBiase L.M., Schade Willis T.M., Wolak E.S., Weber D.J., Rutala W.A.:* Reduction of Healthcare-Associated Infections by Exceeding High Compliance with Hand Hygiene Practices. Emerging Infectious Diseases 2016; 22:1628-1630.
11. *Sutton SVW., Proud DW., Rachui S., Brannan KD.:* Validation of microbial recovery from disinfectants. Journal of Pharmaceutical Science and Technology 2002; 56: 255-266.
12. *Tarka P.:* Hygienic and surgical hand disinfection – European Standards, skin protection of hands. Zakażenia 2010; 6: 14-20 (in Polish).
13. *Tyski S., Bocian E., Grzybowska W.:* Reduction of the neutralization time during antimicrobial activity testing of disinfectants according to European Standards. Rocz Panstw Zakl Hig 2013; 64: 111-115.
14. *Ziółko A., Grzesiowski P.:* Prevention of infection of the operated site. Recommendations according to current medical knowledge 2014. Part 1. The antiseptic of the patient's skin before surgery. Biblioteka Wytycznych i Standardów SHL 2013; 2: 16-28 (in Polish).

Received: 10.08.2017
Accepted: 12.10.2017