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Znaczenie normy PN-EN 16615: 2015 w ocenie aktywności bakteriobójczej preparatów dezynfekcyjnych przeznaczonych do nasączania chusteczek w obszarze medycznym

Importance of the PN-EN 16615: 2015 standard in the assessment of bactericidal activity of disinfectants intended for soaking wipes in medical area

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Norma PN-EN 16615: 2015 jest przeznaczona do określenia parametrów działania preparatów dezynfekcyjnych stosowanych do dezynfekcji powierzchni z działaniem mechanicznym. Uwzględnia ona sposób aplikacji preparatu dezynfekcyjnego na powierzchnię gładką, poddawaną dezynfekcji. Zapewnia prawidłowe wyznaczenie parametrów działania preparatów dezynfekcyjnych stosowanych w formie chusteczek, co przyczynia się do przerwania transmisji zakażeń krzyżowych w obszarze medycznym, szczególnie tych przenoszonych przez powierzchnie dotykowe.

Słowa kluczowe: aktywność bakteriobójcza, preparat dezynfekcyjny, chusteczki, powierzchnie dotykowe

ABSTRACT

Introduction: The PN-EN 16615: 2015 standard is intended to determine the acting parameters of disinfectants used for surfaces disinfection with the wiping technique using wipes. It takes into account the application method of disinfectants on the non porous surfaces to be disinfected. The aim of the study was to assess the compliance of the obtained results of control tests with the limit values set in the PN-EN 16615: 2015 standard and to determine the disinfection parameters of the tested product against *Staphylococcus aureus*.
Method: The disinfectant product tests were carried out using standard wipes described in PN-EN 16615: 2015 standard in four contact times of 1, 5, 10, 15 minutes under clean conditions: 0,3 g / l of bovine albumin solution.
Results: Obtained results of control and validation of the dilution-neutralization method met the criteria of limit values of the PN-EN 16615: 2015 standard. The test product was active against *Staphylococcus aureus* within 15 minutes contact time under clean conditions (reduction > 5,59 lg).

Conclusions: PN-EN 16615: 2015 standard ensures proper determination of acting parameters of disinfectants intended for use in the form of wipes, what contributes to effective disinfection of the surfaces, especially high-touch surfaces, and prevents the occurrence of cross-contamination of other surfaces in the medical area.

Key words: bactericidal activity, disinfectants, wipes, high-touch surfaces

INTRODUCTION

One of the more frequently used surfaces disinfection method is disinfection with mechanical action with using the wiping technique (6, 11). It is made using various types of cleaning tools, of which the wipes are the most widely used. The wipes are supplied by the manufacturers together with the disinfectant or as soaked in a ready-to-use form. Assessment of biocidal activity of this type of disinfectants required the development of new test methods taking into account the method of application of such products to the surface (4, 7, 11,12, 13).

The PN-EN 16615: 2015 standard is intended for the assessment of bactericidal and yeasticidal effectiveness of disinfectants used for surfaces disinfection with the use of a wiping technique in the medical area (6; 7). With this standard, acting parameters of disinfectants are evaluated under conditions simulating their practical application. Testing of the disinfectant may be carried out using standard wipes described by the standard, supplied by the manufacturer together with a disinfecting preparation or soaked in a ready to use form (7).

The test surface simulates a carrier made of PVC, a material most commonly used in hospitals as a floor covering (1, 7). It is the equivalent of a non porous surface on which the preparation intended for disinfection using the wiping technique is distributed in practice. The test method is referred to as the "4-field test", as four squares of 25 cm^2 each are marked on the surface of the carrier, from which the microorganisms are recovered. The suspension of bacteria or *Candida albicans* in an organic interfering substances, is applied to the first test field and fixed to the surface by drying. The action of the disinfection product is checked after wiping the surface of 4 fields with a wipe soaked in the tested product, starting from the first field along the following 3 fields and turning after field 4 to field 1, i.e. returning to the starting point (7). It is a fragment of the semicircle-zigzag movement recommended when performing cleaning and disinfection of surfaces in the medical area (2). This action is aimed at spreading microorganisms along four fields and then return them to the first field. In this way, the parameter of the spread of microorganisms on the surface to be disinfected is assessed. The spreading of microorganisms during the disinfection with mechanical action is observed in the case of disinfecting preparations not showing biocidal efficacy or in the case of cleaning agents. Also size, thickness, composition of materials, layering and degree of absorbency of wipes can influence the capacity of microorganisms spreading on the surfaces (12). The number of microorganisms in individual fields is determined after the contact time of the tested product with the disinfected surface by taking swabs from their surface. The disinfectant can be considered as bactericidal and / or yeasticidal if the reduction of microorganisms on the first field is 5 lg against *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* and 4 lg against *Candida albicans* and the control limits specified in the standard are met (7).

The aim of our research was to introduce the PN-EN 16615: 2015 standard into the scope of laboratory tests within the quality management system by assessing the compliance of the obtained results of control and validation with the limit values set in the standard and determining the disinfection parameters of the tested product against *S. aureus*.

MATERIALS AND METHODS

Test organism: *S. aureus* ATCC 6538 suspension with a density (N) in the range of $1{,}5\times10^9$ cfu/ml do 5×10^9 cfu/ml.

Test surface: PVC carrier with dimensions of 20 cm × 50 cm with 4 marked squares with sides 5 cm × 5 cm at a distance of 5 cm from each other (T1-T2 fields). An identical surface was used for control of sterile distilled water with the addition of Polysorbate 80 (Nw).

Surface for recovery control (Dc0 and Dct): PVC carrier with dimensions of 7 cm × 13 cm with 2 marked squares with sides 5 cm × 5 cm.

Composition of the test preparation: Ethanol – 500 mg/g; N,N-didecyl-N-methyl-poly(oxyethyl)ammonium propionate – 1,1 mg/g.

Standard wipes: 17,5 cm × 28 cm; composition 55% pulp and 45% polyethylenterephthalat (PET) - Tork Premium Sepzial Tücher, Tork Company.

Granite block: dimensions: 12,1 cm × 8,6 cm × 8,6 cm, weight: 2,3 – 2,5 kg; simulates the pressure used when wiping the surface in practical conditions

Neutralizer: Polysorbate 80 – 30 g/l; saponin – 30 g/l; lecithin – 3 g/l in diluent.

Test parameters: product concentration: product ready to use, contact times: 1, 5, 10, 15 minutes, clean conditions: 0,3 g / l of bovine albumin.

Principle of the method: The tests were carried out using the dilution-neutralization method. The wipes were soaked with the test product or in the case of Nw control with the sterile distilled water with the addition of Polysorbate 80. The test surface, the Nw control surface and the recovery control surface (Dc0 and Dct) were infected with the tested suspension of *S. aureus* in the interfering substances solution (final concentration of bovine albumin in suspension – 0,3 g / l), and attached to the surface by drying. The test surface and the Nw control surface were wiped respectively with wipes soaked with the test product and sterile distilled water with the addition of Polysorbate 80, with using standard pressure. After the contact time, *S. aureus* was recovered from the each tested surfaces with moistened cotton swabs. Swab tips were transferred to the neutralizer to abolish action of the test product and to recover *S. aureus* by shaking. After the neutralization time, the growth medium was inoculated with obtained mixture and incubated at 37 °C for 48 hours. In parallel, the same procedure was applied in the case of surfaces intended to recovered *S. aureus* after drying at time t = 0 (Dc0) and after tests and the contact times t = 1, 5, 10, 15 minutes (Dct). The impact of the test conditions and method verification were determined by performing a neutralizer control (control B - absence of toxicity of neutralizer) and validation of the dilution-neutralization method (method validation C) using the validation suspension of *S. aureus* (Nv0) (7).

Calculations: The bactericidal activity of the product in the tested contact times was determined based on the reduction in the T1 field (R_{T1}). R_{T1} is the difference calculated from the number of microorganisms recovered from the surface for recovery control after the tested contact times (Dct) and the number of microorganisms remaining on the surface after the action of the test product (7). The reduction R_{T1} was expressed in a decimal logarithmic scale (lg). The results are the mean of three replications.

RESULTS AND DISCUSSION

In order to assess the conformity of the obtained results of control and validation with the assumptions of the standard PN-EN 16615: 2015, *S. aureus* was selected as one of the 3 test organisms required to determine the bactericidal effect of disinfecting preparations intended to soaking wipes. *S. aureus* is often used in studies of the bactericidal effectiveness of disinfectants as a model organism for Gram-positive bacteria (5, 6). It is characterized by good survival on non-living surfaces in a hospital environment. In the case of methicillin-resistant *S. aureus* (MRSA), the survival range on surfaces until to 7 days and to more than 7 months (3). The survival of *S. aureus* in adverse environmental conditions is important in studies of the biocidal effectiveness of disinfectants because most often the microorganisms are attached to the surface of the carriers by drying at 37 °C (7, 11). In the conducted research, *S. aureus* recovery from carriers intended for drying control (Dc0 and Dct), after the drying process at time t = 0 and after each of the tested contact times (t = 1, 5, 10, 15 minutes) was consistent with the assumptions of the PN-EN 16615: 2015 standard. There was also no effect of the test conditions on *S. aureus* recovery after testing with sterile distilled water with Polysorbate 80 (Nw) and the effect of the toxicity of the used neutralizer (B) on the tested validation suspension (Nv0). The validation of the dilution-neutralization method (C) showed that the used neutralizer abolished the residual action of the tested disinfectant and ensured the correct determination of contact times selected for the study (Table I).

Table I. Control parameters of the dilution-neutralization method and results obtained with the selected *S. aureus* ATCC 6538 test organism at the tested contact times (1, 5, 10, 15 minutes) according to PN-EN 16615: 2015-06.

Control Parameters	Unit	Contact times (min)				Basic limits
		1	5	10	15	
Nv0	cfu/ml	131	121	121	107	from 30 to 160
B	cfu/ml	127	115	115	107	≥0,5×Nv0
C	cfu/ml	121	125	125	118	≥0,5×Nv0
N0	lg	8,25	8,26	8,26	8,27	7,88≤lg≤8,40
Dc0	lg	7,23	7,37	7,36	7,40	6,88≤lg≤8,40
Dct	lg	7,24	7,32	7,37	7,44	6,88≤lg≤8,40
Mean $Nw_{(T2-T4)}$	cfu/25 cm^2	>15767	2283	>13133	1450	>10

Nv0 - validation suspension, B - toxicity control of the neutralizer, C - validation of the dilution-neutralization method, N0 - density of the *S. aureus* suspension at time t = 0; Dc0 - recovery control after drying at time t = 0; Dct - recovery control after the contact time t =1, 5, 10, 15 minutes; Nw (T2-T4) - the average number of *S. aureus* on the fields from T2 to T4.

The test disinfectant intended for soaking the wipes did not show bactericidal activity against *S. aureus* at contact times of 1, 5 and 10 minutes under clean conditions. The bactericidal effect of the wipes was found during 15 minutes contact time, in which the reduction achieved was in accordance with the requirements of PN-EN 16615: 2015 and amounted > 5,59 lg. Wipes soaked with the tested disinfectant did not meet the requirements of European Standards determined for the disinfection of high-touch surfaces, where the activity of the disinfectant on the surface should be achieved during contact times from 1 to 5 minutes (7, 8, 9). However, they met the require-

ments according to the National Institute of Public Health - National Institute of Hygiene methods, in which the contact time for surfaces disinfection can not be longer than 15 minutes (10). Wipes soaked with disinfectant are products most frequently used for surfaces often touched by patients and medical personnel such as bed rails, door handles or bedside tables, computer keyboard (13). For this reason, it is important that they action was in short contact times. The bactericidal activity of disinfectants used for this type of surfaces is also an important factor preventing the spread of bacteria and cross-infection in the medical area (3, 13). In the case of wipes soaked with the tested disinfectant, there was no *S. aureus* spread on the used carriers. The number of bacteria in the T2 to T4 fields ranged from 0 to 5 cfu /25 cm^2 and met the requirements of the standard in each of the contact times studied (Table II).

Table II. Reduction of *S. aureus* ATCC 6538 obtained in the T1 field and the average number of colony forming units in the T2 to T4 fields.

Parameters	Unit	Contact times (min)				Basic limits
		1	5	10	15	
Reduction test field 1 (R_{T1})	lg	3,08	3,73	4,79	>5,59	R≥ 5
Mean T2-T4	cfu/25 cm^2	0	0	0	5	≤ 50

The amount of product released on the test surface during the wiping was similar in all tested contact times and had no effect on its bactericidal activity. The largest amount of product was released to the surface in the test with 5 minutes contact time, in which the required reduction against *S. aureus* was not achieved. The amount of sterile distilled water with the addition of Polysorbate 80 released to the surface at the same pressure was lower than the product tested and ranged from 1.3 g to 1.6 g. The amount of water released on the surfaces ensured the correct distribution of *S. aureus* in the T2 to T4 fields (Table I and III).

Table III. Mean mass of product and of the sterile distilled water with Polysorbate 80 (Nw) released to the surface of the carrier.

Parameters	Unit	Contact times (min)			
		1	5	10	15
Mass of product	g	2,0	2,4	2,2	2,1
Mass of Nw	g	1,3	1,4	1,5	1,6

CONCLUSIONS

1. The results obtained against *S. aureus* in the control tests met the limit values criteria set in the PN-EN 16615: 2015 standard.
2. Wipes soaked with the tested disinfectant achieved the required reduction against *S. aureus* during 15 minutes contact time, under clean conditions.
3. PN-EN 16615: 2015 standard allows for proper determination of acting parameters of disinfecting products intended for soaked of wipes in conditions simulating their practical application, what contributes to the effective disinfection especially of high-touch surface, and prevents the spread of cross-contamination in the medical area.

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