MEDICAL SCIENCE MONITOR

PRODUCT INVESTIGATIONS

e-ISSN 1643-3750
© Med Sci Monit, 2014; 20: 666-673
DOI: 10.12659/MSM.890175

Received: 2013.12.12
Accepted: 2014.01.08
Published: 2014.04.22

Evaluation of mycobactericidal activity of selected chemical disinfectants and antiseptics according to European standards

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDEF 1 Ewa Bocian
BCDE 1 Wanda Grzybowska
ACDEG 1,2 Stefan Tyski

1 Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
2 Department of Pharmaceutical Microbiology, Medical University of Warsaw, Warsaw, Poland

Corresponding Author: Stefan Tyski, e-mail: s.tyski@nil.gov.pl
Source of support: Departmental sources

Background: The history of the investigation of standardized mycobactericidal activity of disinfectants and antiseptics is not very long. There is growing interest among the manufacturers of disinfectants in carrying out research on the antimicrobial activities in accordance with European standards (EN). This research could facilitate the introduction of high-quality disinfectants to the market. The aim of this study was to evaluate the mycobactericidal activity of selected chemical disinfectants and antiseptics used in the medical and veterinary fields.

Material/Methods: This study included 19 products submitted to the National Medicines Institute in Poland for evaluation of mycobactericidal activity. These products contain in their composition active substances belonging to different chemical groups, including aldehydes, alcohols, amines, quaternary ammonium compounds, phenols, guanidine, and oxidizing compounds. This study, conducted according to the manufacturers' description of the preparations, was carried out in accordance with European standards, which also met the Polish standards: PN-EN 14204: 2013, PN-EN 14348: 2006, and PN-EN 14563: 2012.

Results: Tested products for disinfection and antiseptics containing active substances from different chemical groups showed high mycobactericidal activity and met the requirements of the appropriate European standards in most cases. In the case of products containing guanidine and amine compounds, the concentration of active ingredients used in the test and the test conditions specified by the manufacturer did not provide the mycobactericidal activity required by the standards.

Conclusions: Prior to the launch of a new product on the market, it is important to establish the appropriate usage and testing conditions of the preparation, such as its practical concentration, contact time, and environment condition (clean or dirty).

MeSH Keywords: European Union • Anti-Bacterial Agents • Tuberculosis • Antisepsis • Disinfection

Full-text PDF: http://www.medscimonit.com/download/index/idArt/890175

2855 3 — 19

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

The investigation of the mycobactericidal activity of disinfectants and antiseptics has been standardized for several years. The first European standard EN 14204 for testing the mycobactericidal activity of products used in the veterinary field was implemented by the CEN (European Committee for Standardization) in 2004. The standard EN 14563, dedicated to mycobactericidal products used in the medical area, was established in 2008. The laboratory research performed used mycobacterial strains derived from the American Type Culture Collection (ATCC), except for *Mycobacterium tuberculosis*, due to the risk of infection in the laboratory and the slow growth of microorganisms [1]. In addition, the mycobacteria that cause tuberculosis are classified by the US Centers for Disease Control and Prevention (CDC) in Atlanta as category C, and this pathogen can be used as a biological weapon.

According to World Health Organization (WHO), tuberculosis, HIV, and malaria are the most common infectious diseases in the world (one-third of the world population is infected with *Mycobacterium tuberculosis*) [2]. In this context, effective disinfection, in addition to regular immunization, is a significant method for the limitation of mycobacterial infections.

Mycobactericidal activity testing was performed in accordance with European standards (EN) based on the culture of mycobacteria, which means that the test results are known after 21 days. However, the use of the *Mycobacterium terrae* strain, which contains the gfp_m^{2+} gene, causes fluorescence of living mycobacteria cells and can reduce the detection time to 15 days [3,4].

There is growing interest among manufacturers of disinfectants and antiseptics in conducting tests of mycobactericidal activity in accordance with ENs, which will make the presence of high-quality products on the market possible.

The aim of this study was to evaluate the mycobactericidal activity against 2 mycobacterial species, *Mycobacterium avium* and *Mycobacterium terrae*, or the activity only against *Mycobacterium tuberculosis*, of selected chemical disinfectants and antiseptics used in the medical and veterinary areas in accordance with relevant European standards [5–7].

The Department of Antibiotics and Microbiology at the National Medicines Institute, Warsaw, Poland, has investigated the antimicrobial effectiveness of antiseptic and disinfectant products for many years. Recently, we compared selected commercial mouthwash and disinfection products [8]. Our department is certified by the European Directorate for the Quality of Medicines (EDQM) for microbiological tests carried out in accordance with ISO/EN 17025 and possesses the accreditation of the Polish Centre for Accreditation (No. AB 774) for microbiological testing of disinfectants and antiseptics, according to several ENs.

Material and Methods

Products

These studies included 19 products submitted to the National Medicines Institute in Poland for the evaluation of mycobactericidal activity. These products contain active substances that belong to different chemical groups, including aldehydes, alcohols, amines, quaternary ammonium compounds, guanidine, phenols, and oxidizing compounds (Table 1).

Standards

Investigations, according to the manufacturers' description of the products, were carried out in accordance with European standards, which also met the Polish standards: PN-EN 14204: 2013 (EN 14204: 2012): Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants used in the veterinary area – Test method and requirements (phase 2, step 1) [5]; PN-EN 14348: 2006 (EN 14348: 2005): Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants – Test method and requirements (phase 2, step 1) [6]; and PN-EN 14563: 2012 (EN 14563: 2008): Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area – Test method and requirements (phase 2, step 2) [7].

Products recognized as mycobactericidal should be active against both *Mycobacterium avium* and *Mycobacterium terrae* mycobacterial species. Product activity against *Mycobacterium tuberculosis* is determined in accordance with the recommendations of the European Committee for Standardization (CEN), only to strain *M. terrae*.

The product meets the requirements of the PN-EN 14563: 2012 when after 60 min of contact time of the product and mycobacteria suspension in the appropriate temperature (10°C or 20°C), it demonstrates at least a decimal log reduction in counts of 4 (reduction ≥4 log cfu/mL) of both organisms (mycobactericidal activity) or only against *M. terrae* (tuberculocidal activity).

Depending on the application of the products, interfering substance were used: clean conditions were simulated by 0.3 g/L bovine serum albumin /BSA/ (according to EN 14348 and EN 14563), or by a solution of BSA 3.0 g/L (according to EN 14204)

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 1. Characteristic of disinfectants and antiseptics tested in this study.

Name of product, form	Manufacturer	Composition of active substances
Aldesan E, liquid	Septoma, Poland	Glutaraldehyde 2%, ethanol 25%
Aldizol, liquid	Septoma, Poland	o-phenylphenol 7.0%, 4-chloro-3-methylphenol 4.5%, glutaraldehyde 4.0%
Chlor-Clean, tablets	Guest Medical Ltd., Great Britain	Sodium dichloroisocyanurate
Desisoft Ytdesinfektion, liquid	Rekal Svenska AB, Sweden	Polyhexamethylenguanidine hydrochloride 0.35% w/w
Lysoformin Plus-Schaum, liquid	Lysoform, Germany	N-(3-aminopropyl)-N-dodecylopropano -1,3-diamine 0.46 g, didecyl dimethyl ammonium chloride 0.10 g, polyhexamethylene biguanidine 0.18 g/100 g
OneMed Easydes, liquid	Farmos Ltd., Finland	55–60% ethanol, t-butanol <2%, ammonium chloride <0.5%, alkylamine <0.5%
Rafasept, liquid	Septoma, Poland	o-phenylphenol 12.5%, 4-chloro-3-methylphenol 3.0%
Septyl Amyco, powder	Septoma, Poland	o-phenylphenol 12.0 g, of 4-chloro-3-methylphenol 4.0 g, 2-benzyl-4-chlorofenol 1g/100 g
Septyl R, liquid	Septoma, Poland	Chlorocresol (4-chloro-3-methylphenol) 1.0 g /100g
Soft Care Des E Spray, liquid	JohnsonDiversey UK Ltd.	Ethanol 715 g/kg
ST4, powder	Steril-4 S.R.L., Italy	Sodium percarbonate >40 g, TAED >25 g/100 g
Steril C, powder	Steril-4 S.R.L., Italy	Sodium perborate, TAED
Steril-Ser, powder	Steril-4 S.R.L., Italy	TAED > 25 g/100 g
Synrol ALC, liquid	Synpeko, Poland	Ethanol 70%, isopropanol 15%
Synrol PAA10, liquid	Synpeko, Poland	Peracetic acid 10%, hydrogen peroxide 13%, acetic acid 10%
Synsept AG, liquid	Synpeko, Poland	Glutaraldehyde 12%, benzalkonium chloride 3%, didecyl dimethyl ammonium chloride 6%
Synsept BOR, powder	Synpeko, Poland	Sodium perborate 24%, TAED – 12%
Synsept PAA, liquid	Synpeko, Poland	A: peracetic acid 3.5%; B: hydrogen peroxide 20% (5% A + 5% B)
Virusolve + EDS, liquid	Amity International, Great Britain	Alkilotriamine 12 g, 2-aminoethanol 8 g

and dirty conditions, 3.0 g/L BSA, 3.0 mL/L sheep erythrocytes (according to EN 14348 and EN 14563) or yeast extract 10 g/L, BSA 10 g/L (according to EN 14204).

Strains

In the studies performed according to the PN-EN 14348 and PN-EN 14563, 2 reference strains of mycobacteria – *Mycobacterium avium* ATCC 15769 and *Mycobacterium* terrae ATCC 15755 – were used. In tests performed according to PN-EN 14204, only the strain *M. avium* ATCC 15769 was used.

Results

The majority of products (17/18) for use in the medical field were tested in accordance with EN 14348. One product designed for use in the veterinary field was evaluated according to EN 14204. Both of these standards are to be tests of phase 2 step 1 (suspension method) in which the products are tested under simulated conditions by the introduction of inorganic loads (hard water to dilute the product) and organic loads (albumin, albumin + erythrocytes, or albumin + yeast extract). Only 4 studies were conducted in accordance with EN 14563

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

– the phase 2 step 2, in which a test suspension of mycobacteria in a solution of interfering substances was spread on a glass carrier (glass slides).

The majority of tested products (16 out of 19) showed mycobactericidal activity in accordance with the applicable standard under the selected conditions of the study (Tables 2 and 3). Only 1 product, Desisoft Ytdesinfektion, did not present what was required by the appropriate standard of mycobactericidal activity in undiluted form, even when the longest contact time (60 min) was applied. The study was conducted at the request of the manufacturer; however, the mycobactericidal activity was not declared on the product's label. The other 2 products – Lysoformin Plus-Schaum and Virusolve + EDS – showed activity against only 1 of the mycobacteria strains – *M. avium* (Tables 2 and 3). Proper selection of the test conditions (concentration, contact time, interfering substance) allows for determination of the mycobactericidal activity in specific conditions of use. Table 3 presents the results of the activity of the product Virusolve + EDS, after 4 contact exposures of 5, 15, 30, and 60 min, examined in carrier test under simulated clean and dirty conditions. There was a significant increase in the activity of the product against *M. avium*, from about 2.3 log after 5 min to more than 4.9 log after 60 min. The extent of organic load had no effect on mycobactericidal activity. The prolongation of contact time of this product with *M. terrae* from 5 to 60 min did not cause appropriate activity (reduction minimum 4 log), although with increasing contact time, the product caused reduction of tested mycobacteria from a value of below 1.4 log to about 3.0 log. The manufacturer of the product (Virusolve + EDS) declared mycobactericidal activity at a 2.5% concentration after 5 min of contact time. The results in Table 3 show that this product, at a concentration of 2.5%, is active against *M. avium* only after 30 min of contact time, but in the case of *M. terrae*, the 4-log reduction was not achieved, even after 60 min of exposure to the agent.

Discussion

Mycobactericidal activity of disinfectants and antiseptics depends on chemical compositions, concentrations of use, durations of contact time, and organic loads.

Aldehydes, phenols, quaternary ammonium compounds, and polyalkylamines are commonly used as detergents/disinfectants in the disinfection procedure. Because of the toxicity of aldehydes and phenols, new disinfectant formulations are being developed. The antimicrobial activity of amine derivatives has been studied for some time.

Amine-based disinfectants have become quite popular for the disinfection of surgical instruments and other medical devices. Korsolex® AF (15.6% dodecyl-bis-propylene triamine and 5.1% lauryl propylene diamine) was studied by Hernandez et al. [9]. The mycobactericidal and tuberculocidal activities of Korsolex® AF against *M. tuberculosis* H37 Rv ATCC 25618, *Mycobacterium kansasii* ATCC 12478, *M. chelonae* ATCC 35752, and a MAI (*M. avium – M. intracellulare*) clinical strain were determined using quantitative bacteria suspension and carrier tests. The effects of organic load and hard water were also considered. Korsolex® AF had acceptable efficacy against several species of mycobacteria in quantitative suspension and carrier tests at a concentration of 2% with an exposure time of 30 min. One of the 4 strains – the MAI clinical strain – was the least sensitive: the reduction in number of mycobacteria was about 4.6 log, while for the other strains the reduction rate was more than 5 log.

Glutaraldehyde is widely used as the active ingredient in high-level disinfectants for heat-sensitive, semi-critical medical instruments, including soft endoscopes or bronchoscopes, which may be contaminated with different strains of bacteria, including mycobacteria, after usage. Flexible fiber-optic bronchoscopy has become accepted as a safe tuberculosis diagnostic and therapeutic procedure and it is typically well tolerated by the patient. However, the transmission of *Mycobacterium tuberculosis*, atypical mycobacteria, and other pathogens between patients undergoing bronchoscopy has been reported due to improper cleaning and disinfection procedures [9].

Miner et al. [10] demonstrated that products containing concentrations of ≤20% w/w isopropanol and ≤8% potassium acetate in combination with ≤3.5% w/w glutaraldehyde at alkaline pH values killed 6 log of mycobacteria – *Mycobacterium bovis* var BCG, *M. terrae* ATCC 15755, and glutaraldehyde-resistant *M. chelonae var abscessus* ATCC 14472 – within 10 min at 20°C.

In our study, products containing glutaraldehyde (Aldesan E, Aldizol, and Synsept AG) have also been found to be effective against mycobacteria (i.e., they meet the requirements of EN 14348).

Much work has been devoted to the study of the mycobactericidal activity of products containing ortho-phthalaldehyde (OPA), glutaraldehyde (GTA), and other dialdehydes.

Fraud et al. [11] showed high mycobactericidal activity of aldehydes (OPA) at 0.5% (v/v) unadjusted pH 6.5 and pH 8, under both clean and dirty conditions. Test organisms consisted of glutaraldehyde (GTA)-sensitive strains of *Mycobacterium chelonae* NCTC 946, *M. abscessus* NCTC 10882, two GTA-resistant *M. chelonae* strains, and *M. terrae* NCTC 10856 (a proposed *M. tuberculosis* surrogate). All mycobacterial reference strains used in the suspension test under clean conditions were very sensitive to 0.5% alkaline OPA, resulting in a log reduction factor of 5 after 1 min exposure or after 10 min with *M. abscessus* NTCC 10882.

This work is licensed under a Creative Commons
Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 2. Results of mycobactericidal activity of tested disinfectants and antiseptics; assays carried out in accordance with European Standards.

Product	Standard	Test conditions – according to manufacturers order	Result (log of mycobacteria reduction)
Aldesan E	EN 14348	Undiluted, 60 min, dirty condition	M.a. >6.27; M.t. >6.22
Aldizol	EN 14348	1.75%, 15 min, clean condition, 2.5%, 15 min dirty condition	M.a. >6.54; M.t. >6.21
Chlor-Clean	EN 14348	1000 ppm, 15 min, clean condition	M.a. – 4.59; M.t. – 4.40
		10000 ppm, 15 min, dirty condition	M.a. >6.20; M.t. >6.30
Desisoft Ytdesinfektion	EN 14348	Undiluted, 5, 30 i 60 min, clean and dirty condition	M.a. <2.66; M.t. <2.42
Lysoformin Plus-Schaum	EN 14348	Undiluted, 15 min, clean condition	M.a. – 4.19; M.t. <3.09
		Undiluted, 15 min, dirty condition	M.a. – 4.09; M.t. <3.09
OneMed Easydes	EN 14348	Undiluted, 1 min, dirty condition	M.a. >6.55; M.t. >6.21
		Undiluted, 5 min, dirty condition	M.a. >6.55; M.t. >6.26
Rafasept	EN 14348	1.5%, 15 min, dirty condition	M.a. >6.55; M.t. >6.03
Septyl Amyco	EN 14348	2%, 30 min; 2.25%, 15 min; 2.75% 15 min, clean and dirty condition	M.a. >6.55; M.t. >6.03
Septyl R	EN 14348	1.5%, 15 min, clean and dirty condition	M.a. >6.54; M.t. >6.13
Soft Care Des E Spray	EN 14348	Undiluted, 30 s, clean condition	M.a. – 5.81; M.t. – 5.71
		Undiluted, 30 s, dirty condition	M.a. – 5.70; M.t. – 5.82
ST4	EN 14204	0.5%, 10 min, high levels soiling	M.a. <2.89
		1%, 10 min, high levels soiling	M.a. <2.89
		2%, 10 min, high levels soiling	M.a. <3.60
		0.5%, 25 min, high levels soiling	M.a. <2.89
		1%, 25 min, high levels soiling	M.a. <2.89
		2%, 25 min, high levels soiling	M.a. <4.20
	EN 14563	0.5%, 10 min, dirty conditions	M.a.>5.11
		1%, 10 min, dirty conditions	M.a.>5.11
		2%, 10 min, dirty conditions	M.a.>5.11
		0.5%, 25 min, dirty conditions	M.a.>5.11
		1%, 25 min, dirty conditions	M.a.>5.11
		2%, 25 min, dirty conditions	M.a.>5.11
Steril C	EN 14348	1%, 20 min, clean condition	M.a. <3.17; M.t. – 4.02
		1%, 20 min, dirty condition	M.a. <3.17; M.t. <2.96
		1.5%, 20 min, clean condition	M.a. – 5.95; M.t. >6.33
		1.5%, 20 min, dirty condition	M.a. – 4.61; M.t. >6.33
Steril-Ser	EN 14348	1%, 10 and 15 min, dirty condition	M.a. >6.43; M.t. >6.07
	EN 14563	1%, 10 and 15 min, dirty condition	M.t. >5.04
Synrol ALC	EN 14348	Undiluted, 1 min, clean condition	M.a. >6.48; M.t. >6.13
	EN 14563	Undiluted, 15 min, clean condition	M.a. >4.59; M.t. <2.87
Synrol PAA10	EN 14348	2%, 15 min, dirty condition	M.a. >6.48; M.t. >6.13
Synsept AG	EN 14348	4%, 60 min, dirty condition	M.a. >4.58; M.t. >4.02
Synsept BOR	EN 14348	2%, 15 min, dirty condition	M.a. >4.92; M.t. >6.13
Synsept PAA	EN 14348	5%A + 5%B, 10 min, dirty condition	M.a. >6.48; M.t. >6.13

Test conditions: concentration, contact time, clean or dirty conditions, M.a. – *M. avium*, M.t. – *M. terrae*.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Table 3. Detail results of mycobactericidal activity of Virusolve + EDS product carried out in accordance with EN 14563.

Microorganism	Contact time (min)	Logarithm of reduction in number of mycobacterial cells in the samples of 2,5% concentration			
		Clean condition 0.3 g/L bovine albumin		Dirty condition 3.0 g/L bovine albumin 3.0 mL/L sheep erythrocytes	
		I test	II test	I test	II test
M. avium ATCC 15769	5	2.26	2.27	2.30	2.35
	15	3.91	3.92	3.72	3.87
	30	4.47	4.59	>4.89	>4.92
	60	>4.89	---	>4.89	---
M. terrae ATCC 15755	5	<1.37	<1.36	<1.37	<1.36
	15	<1.63	<1.71	<1.19	<1.26
	30	<1.63	<1.71	1.5	1.57
	60	3.31	2.99	3.16	3.06

In the carrier test, in which discs of polypropylene (the material of which endoscopes are constructed) were covered with biofilm formed from a mixture of mycobacteria with sodium alginate, a high degree of bacterial reduction was achieved (≥5 log) for a 0.5% w/v solution of OPA, but after a longer contact time (30–60 min) and only under clean conditions [12].

Mycobactericidal activity of Cidex (2% glutaraldehyde) and Cidex OPA (0.55% ortho-phthalaldehyde) was evaluated with *M. smegmatis* ATCC 19420 and with clinical isolates of *M. fortuitum*, *M. abscessus,* and *M. tuberculosis* H37Rv (apart from the clinical strain of *M. chelonae*). Cidex and Cidex OPA were effective against tested organisms, showing greater than a 5-log reduction in colony-forming units (CFU) after 5 min of exposure under both clean and dirty conditions [13].

In the past decade, high-level disinfectants based on glutaraldehyde have become widely used in disinfection because of their mycobactericidal activity. However, disinfection with glutaraldehyde has recently been of some concern because of its toxicity, skin and respiratory sensitizing of hospital staff, and the emergence of resistant mycobacteria. This agent also selects for strains of several microorganisms with a decreased susceptibility to 2% alkaline glutaraldehyde, such as *Mycobacterium chelonae*.

Several studies have been performed to assess possible alternatives to glutaraldehyde.

The *in vitro* and *in vivo* studies conducted by Hernandez et al. [14,15] showed the equivalency of products for disinfection of bronchoscopes, containing 2% glutaraldehyde (Cidex) and 0.26% peracetic acid (Perasafe). Both preparations caused a reduction in the number of mycobacteria of more than 5 log after 20 to 30 min, under both clean and dirty conditions.

In our studies, products containing peracetic acid also caused high reduction ratios (over 6 log) of both mycobacterial strains of *M. terrae* and *M. avium* after 10 to 15 min of contact time in a study conducted in dirty conditions.

Chlorine dioxide may be an alternative to aldehydes in disinfection of soft endoscopes and other medical equipment.

In the carrier test according to the modified method of prEN 14563: 2005, Hernandez et al. [16] studied the activity of the product 'Tristel Sporicidal Wipes'. This system is composed of 2 components: a wipe that is saturated with a mixture of organic acids, preservatives, buffers, and corrosion inhibitors, and a bottle containing a sodium chlorite-based foam. Prior to testing, the wipes were prepared by squirting foam onto the wipe and then scrunching it by hand to mix the 2 components of the product to activate the disinfectant. The chlorine dioxide concentration in the activated wipe was 200 ppm. The results showed that the chlorine dioxide wipes were mycobactericidally active against *M. avium* ATCC 15769 with 30 s contact time with mechanical action and in 60 s without mechanical action, under both clean and dirty conditions.

In our suspension studies (EN 14348), chlorine-based disinfectant (Chlor-Clean) showed activity against both strains of mycobacteria *M. avium* and *M. terrae,* compatible with the standard. Increasing the chlorine concentration of use from 1000 ppm to 10 000 ppm, despite the changing conditions from clean to dirty at the same contact time; 15 min resulted in a significant increase in the degree of reduction of bacteria, from approximately 4 log to over 6 log.

An example of a different, new biocidal substance from the oxidizing agents group is 2-butanone peroxide, which is proposed

This work is licensed under a Creative Commons
Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

for use in antiseptic and disinfectant products dedicated for skin disinfection and for the disinfection of instruments and surfaces in a hospital environment. Garcia-de-Lomas et al. [17] tested the biocidal activity of different concentrations of 2-butanone peroxide against different microorganisms: bacteria, spores, fungi, viruses, and also mycobacteria, including *M. terrae* ATCC 15755. Mycobactericidal activity was assessed following the suspension method described in EN 14348. It showed a degree of reduction of approximately 7 log at a 0.5% solution, after 60 min of contact time at 20°C. Parallel toxicity tests were conducted. Toxicity assessment showed negative results in the acute dermal irritation test, acute eye irritation test, and acute oral toxicity test [17]. The results allow for the recognition of 2-butanone peroxide as an active ingredient suitable for use in the new formulation of antiseptics and disinfectants.

Taking into account the biocidal efficacy of disinfectants, as well as security to users and the environment and compatibility with disinfected materials, a new formulation based on hydrogen peroxide (accelerated hydrogen peroxide, AHD) was developed [18,19]. It contains very low levels of certain food-grade anionic and non-ionic surfactants, which act in synergy with hydrogen peroxide to produce the desired microbicidal activity. Omidbakhsh and Sattar [18] investigated the activity of such a product containing 0.5% hydrogen peroxide against mycobacteria: *M. bovis*, BCG strain and *M. terrae* ATCC 15755. The resulting degree of reduction after a contact time only 5 min at 20°C for these strains was over 6 log in the presence of 5% serum.

Our results confirmed the efficacy of products containing hydrogen peroxide (Synrol PAA10 and Synsept PAA) against mycobacteria *M. avium* and *M. terrae*. The complex composition of the products should also be considered, as they also include other active substances.

Disinfecting products that contain substances from the group of quaternary ammonium compounds do not exhibit effective biocidal activity against mycobacteria. Bello et al. [13] conducted studies with 2 disinfectants – Gerdex and K-ller – which both contain the quaternary ammonium compound dimethyl benzyl lauryl ammonium bromide in concentrations of 10% and 0.16%, respectively. Gerdex and K-ller caused only a 2-log cell reduction of *M. tuberculosis* H37Rv, *M. abscessus,* and *M. chelonae*, even after 60 min of contact time under both clean and dirty conditions. The products tested in our study (Lysoformin Plus-Schaum, OneMed Easydes and Synsept AG), in addition to quaternary ammonium compounds, also contain other active ingredients. Only the product Lysoformin Plus Schaum showed no reduction in accordance with EN 14348 of *M. terrae* cells, even under clean conditions after 15 min of contact time (<3.1 log), but in the case of a *M. avium* the degree of reduction was on the border of the requirements according to standard (approximately 4.1 log).

Conclusions

The tested products for disinfection and antisepsis contain active substances from different chemical groups: aldehydes, alcohols, quaternary ammonium compounds, phenolic compounds, and oxidizing agents. In most cases they showed mycobactericidal activity that complied with European standards. In the case of products that contain guanidines and amine compounds, the concentration of active ingredients used and the test conditions specified by the client that ordered the test did not show the mycobactericidal activity required by the standards. Prior to the introduction of the product to the market, it is important to establish the appropriate conditions for the use of the product, such as the concentration to use, contact time, and clean or dirty conditions.

References:

1. Griffiths PA, Babb JR, Fraise AP: *Mycobacterium terrae*: a potential surrogate for *Mycobacterium tuberculosis* in a standard disinfectant test. J Hosp Infect, 1998; 38: 183–92
2. Global tuberculosis report 2012, WHO. Available from: *http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf*
3. Steinhauer K, Eschenbacher I, Radischat N et al: Rapid evaluation of the mycobactericidal efficacy of disinfectants in quantitative carrier Test EN 14563 by using fluorescent *Mycobacterium terrae*. Appl Environ Microbiol, 2010; 76: 546–54
4. Zafer AA, Taylor YE, Sattar SA: Rapid screening method for mycobactericidal activity of chemical germicides that uses *Mycobacterium terrae* expressing a green fluorescent protein gene. Appl Environ Microbiol, 2001; 67: 1239–45
5. Standard PN-EN 14204: 2013 (EN 14204: 2012): Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants used in the veterinary area – Test method and requirements (phase 2, step 1)
6. Standard PN-EN 14348: 2006 (EN 14348: 2005): Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants – Test method and requirements (phase 2, step 1).
7. Standard PN-EN 14563: 2012 (EN 14563: 2008): Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area – Test method and requirements (phase 2, step 2)
8. Tyski S, Bocian E, Mikucka A, Grzybowska W. Antibacterial activity of selected commercial products for mouth washing and disinfection, assessed in accordance with PN-EN 1040. Med Sci Monit, 2013; 19: 458–66
9. Hernandez A, Martro E, Matas L et al: Mycobactericidal and tuberculocidal activity of Korsolex® AF, an amine detergent/disinfectant product. J Hosp Infect, 2005; 59: 62–66
10. Miner N, Harris V, Cao TD et al: Aldahol high-level disinfectant. Am J Infect Control, 2010; 38: 205–11
11. Fraud S, Maillard J-Y, Russell AD: Comparison of the mycobactericidal activity of ortho-phthalaldehyde, glutaraldehyde and other dialdehydes by a quantitative suspension test. J Hosp Infect, 2001; 48: 214–21

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

12. Shackelford JCN, Hanlon GW, Maillard J-Y: Use of a new alginate film test to study the bactericidal efficacy of the high-level disinfectant ortho-phthaldehyde. J Antimicrob Chemother, 2006; 57: 335–38
13. Bello T, Rivera-Olivero IA, de Waard JH: Inactivation of mycobacteria by disinfectants with a tuberculocidal label. Enferm Infect Microbiol Clin, 2006; 24: 319–21
14. Hernandez A, Martro E, Matas L, Ausina V: *In-vitro* evatuation of Perasafe compared with 2% alkaline glutaraldehyde against Mycobacterium spp. J Hosp Infect, 2003; 54: 52–56
15. Hernandez A, Martro E, Puzo C et al: In-use evaluation of Perasafe compared with Cidex in fibreoptic bronchoscope disinfection. J Hosp Infect, 2003; 54: 46–51
16. Hernandez A, Carrasco M, Ausina V: Mycobactericidal activity of chlorine dioxide wipes in a modified prEN 14563 test. J Hosp Infect, 2008; 69: 384–88
17. Garcia-de-Lomas J, Lerma M, Cebrian L et al: Evaluation of the *in-vitro* cidal activity and toxicity of a novel peroxygen biocide: 2-butanone peroxide. J Hosp Infect, 2008; 68: 248–54
18. Omidbakhsh N, Syed A, Sattar SA: Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. Am J Infect Control, 2006; 34: 251–57
19. Syed A, Sattar SA, Adegbunrin O, Ramirez J: Combined application of simulated reuse and quantitative carrier tests to assess high-level disinfection: Experiments with an accelerated hydrogen peroxide-based formulation. Am J Infect Control, 2002; 30: 449–57

This work is licensed under a Creative Commons
Attribution-NonCommercial-NoDerivs 3.0 Unported License

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]