Health Security
Volume 14, Number 4, 2016 Mary Ann Liebert, Inc.
DOI: 10.1089/hs.2016.0003

Biocidal and Sporicidal Efficacy of Pathoster® 0.35% and Pathoster® 0.50% Against Bacterial Agents in Potential Bioterrorism Use

Antonio Candeliere, Emanuele Campese, Adelia Donatiello, Stefania Pagano, Michela Iatarola, Francesco Tolve, Leonardo Antonino, and Antonio Fasanella

The use of products that can neutralize or significantly reduce the microbial load and that are not harmful to human health and the environment represents a milestone in the fight against the spread of infectious diseases. Peracetic acid, besides being an excellent sterilizing and sporicidal agent, is harmless to humans and the environment when it is used in a common dosage. However, the high costs and loss of efficacy of the product very quickly after its reconstitution limit its use. We evaluated the efficacy and stability of 2 commercial products, based on stabilized peracetic acid (Pathoster® 0.35% and Pathoster® 0.50%) used against spores of *Bacillus anthracis* and spores of *Bacillus cereus* and vegetative forms of *Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis*, *Brucella abortus,* and *Brucella melitensis.* The efficacy tests were based on the direct contact of the products with a standard suspension of the bacteria. The stability of the products was defined as the period of time during which the biocidal and sporicidal properties remained unchanged. The limit of effectiveness was the period after which the product was unable to exert a complete sterilization after a contact of 5 minutes with at least 1 of the 8 bacteria used in this work. Both formulations showed good efficacy against the microorganisms used in the study, confirming the utility of peracetic acid as a sterilizing product. After the reconstitution, Pathoster® 0.35% was stable until 16 ± 1 days, while Pathoster® 0.50% was stable until 24 ± 1 days. The formulations used in this study showed good performance and a significant stability of peracetic acid.

One of the strategic issues in the fight against the spread of bacterial and viral agents is the use of substances that are able to neutralize the pathogenic environment load and that do not have harmful effects on human health and the environment.[1,2] The most indicative parameter of the effectiveness of a sterilizing product is its ability to inactivate bacterial spores and, in particular, those of Bacilli and Clostridia.[3,4] In spore-forming bacterial

Antonio Candeliere, who has a master's degree in chemistry, is responsible for R&D, and Leonardo Antonino, who has a master's degree in chemical engineering, is responsible for production; both at Cerichem Biopharm srl, Cerignola FG, Italy. Antonio Fasanella, DVM, PhD, is Director of the National Reference Center for Anthrax and General Director of Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy. Emanuele Campese, who has a master's degree in biology, and Adelia Donatiello, who has a master's degree in biotechnology, are Biologist Researchers; Stefania Pagano, DVM, is a Researcher; and Michela Iatarola and Francesco Tolve are Laboratory Technicians; all at the National Reference Center for Anthrax, Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy.

agents, in fact, those belonging to the genus *Bacillus* produce spores that are among the most durable in nature. *Bacillus anthracis*, for example, is able to produce spores that can survive in the external environment and under adverse conditions for many decades. The strong lethality for humans, the ease with which it can produce large amounts of spores, and the ability of spores to survive for many years with their virulence unaltered make the *Bacillus anthracis* bacteria one of the most studied as a potential biological weapon.[5] Often in the past, several substances have been used against bacterial agents, viruses, and protozoa, but these substances were highly toxic to humans and harmful to the environment. An example of these substances, widely used for disinfecting medical instruments, is glutaraldehyde, which, although it is an effective sporicidal agent, has tissue-damaging action with a direct action on proteins involved in cell differentiation and on DNA as well as a potential carcinogenic effect.[6]

Compared to glutaraldehyde, peracetic acid has a similar and sometimes better biocidal efficacy.[3,7] Specifically, a contact time of 5 minutes is recommended for the destruction of vegetative forms of some bacteria and viruses (HBV, HIV), while for sporicidal activity against bacilli, a contact time of 10 minutes is needed using solutions of peracetic acid with a concentration of 0.35% and about 10-15 minutes of contact for solutions of peracetic acid with a concentration of 0.09%.[7]

Peracetic acid has the advantage of remaining effective even in the presence of organic residues, and it may also decompose into nontoxic and nonmutagenic substances (acetic acid and oxygen).[3] Peracetic acid has an excellent sterilizing action after short-time contact, it is a strong oxidant, and its oxidation potential is higher than that of chlorine and chlorine dioxide.[8-10] It is soluble in water and in polar organic solvents, but unfortunately the diluted solutions of peracetic acid are very unstable: A solution of peracetic acid with a concentration of 1% loses half of its effectiveness in about 6 days.[4] To extend the time of stability, peracetic acid should be stored at ordinary temperatures, preferably in a cool place, and it should remain inside the original container.

Another way to extend the stability of peracetic acid is to add it to the solution of a chemical compound such as hydrogen peroxide. The stability of the peracetic acid can be affected by glass and by many types of plastics; peracetic acid can cause deterioration of some materials made of vinyl formulations commonly used as gaskets, and it can attack natural and synthetic rubbers.[11] Pure aluminum, stainless steel, and tin-plated iron are resistant to peracetic acid, while normal steel, galvanized iron, copper, brass, and bronze are susceptible to corrosion reaction.[12,13]

The germicidal properties of peracetic acid were reported in the early 1900s by Freer and Novy (1902), who noted "the excellent disinfecting and sterilizing action of peracetic acid at ambient and cold temperatures," but it is thanks to the improvement of the industrial production process that peracetic acid has become a consumer product.[4,14] Hutchings and Xezones (1949) showed that peracetic acid turns out to be the most effective of 23 germicides tested against spores of *Bacillus thermoacidurans*.[15] Greenspan and MacKellar (1951) defined bactericidal activity at a concentration of 0.001%, fungicidal activity at a concentration of 0.003%, and sporicidal activity at a concentration of 0.3%.[16]

The disinfectant activity of peracetic acid is based on the release of active oxygen.[17] It is assumed that peracetic acid either interrupts the chemiosmotic function of the cytoplasmic membrane and therefore the transport through the membrane, or it causes a rupture of the cell wall.[18,19] Its action as a denaturing agent of proteins could explain its properties as a sporicidal and ovicidal agent.[4] In addition, at the intracellular level, peracetic acid may oxidize the essential biochemical enzymes interfering with biochemical pathways and interfering with the active transport across membranes that alter the levels of intracellular solute.[13]

It has been verified that peracetic acid acts on the basis of the DNA molecule.[20] It has been recently demonstrated that the sporicidal activity of peracetic acid is carried out through specific activity against the receptors of germination GerB and GerK.[21] The products of decomposition of peracetic acid are acetic acid, hydrogen peroxide, oxygen, and water, and there are 3 reactions in which peracetic acid degrades in an aqueous solution: spontaneous decomposition, hydrolysis, and the decomposition of transition metal-catalyzed.[22-27] The pH range, which can fluctuate from 5.5 to 8.2, is linked to the spontaneous decomposition in acetic acid and oxygen.[22]

In this article, we evaluate the biocidal and sporicidal efficacy and the stability of 2 commercial products containing 0.35% and 0.50% stabilized peracetic acid (Pathoster® 0.35% and Pathoster® 0.50%); these products have been tested against spores of *Bacillus anthracis* and *Bacillus cereus* and against vegetative forms of *Yersinia pestis, Burkholderia mallei, Burkholderia pseudomallei, Francisella tularensis, Brucella abortus,* and *Brucella melitensis*. The aim was to verify the effectiveness and stability of the 2 products on a range of spore-forming and non-spore-forming bacteria, with particular reference to bacteria named by the Centers for Disease Control and Prevention (CDC) as potential bioterrorism agents.

Materials and Methods

Bacteria

The effectiveness and stability of Pathoster® 0.35% and Pathoster® 0.50% against 8 bacteria have been verified (Table 1).

Bacillus anthracis

Bacillus anthracis is the agent of anthrax. Spores of the pathogen strain A0843 were produced on solid medium for

Table 1. Bacterial Strains and Microbial Load Used in This Work

Bacterial Strain	*Reference*	*Medium*	*N: Amount of Bacteria in Suspension*
Bacillus anthracis	A0843	5% sheep blood agar	$5.2\times10^8\pm20\%$ spores CFU/ml equal to $8.6\leq\log N_{anthrax}\leq8.8$
Bacillus cereus	ATCC 10876	5% sheep blood agar	$6.7\times10^8\pm20\%$ spores CFU/ml equal to $8.7\leq\log N_{cereus}\leq8.9$
Yersinia pestis	NCTC02028	CIN agar	$2.1\times10^9\pm20\%$ CFU/ml equal to $9.2\leq\log N_{pestis}\leq9.4$
Burkholderia mallei	ATCC23344	5% sheep blood agar	$4.9\times10^9\pm20\%$ CFU/ml equal to $9.6\leq\log N_{mallei}\leq9.8$
Burkholderia pseudomallei	A101-10	Ashdown LR	$3.2\times10^9\pm20\%$ CFU/ml equal to $9.4\leq\log N_{pseudomallei}\leq9.6$
Francisella tularensis ssp. *tularensis*	SCHU S4	CHAB-A	$2.6\times10^9\pm20\%$ CFU/ml equal to $9.3\leq\log N_{tularensis}\leq9.5$
Brucella abortus	B19 Vaccine strain	Brucella agar	$2.3\times10^9\pm20\%$ CFU/ml equal to $10.2\leq\log N_{abortus}\leq10.4$
Brucella melitensis	RKI 16 M	Brucella agar	$1.5\times10^9\pm20\%$ CFU/ml equal to $10.1\leq\log N_{melitensis}\leq10.3$

sporulation. The spores were collected after we verified that the level of sporulation was about 95%. The suspension was collected in saline solution 0.9%, and then it was incubated at 56°C for 30 minutes in order to remove all the vegetative forms. After incubation, the spores were washed 3 times in saline solution 0.9%. The stability of the solution containing spores has been verified by plate count carried out every 2 weeks for 3 months. In the tests, we used a stabilized suspension $N_{anthrax}$ containing $5.2\times10^8\pm20\%$ spores CFU/ml equal to $8.6\leq\log N_{anthrax}\leq8.8$.

Bacillus cereus

Spores of the strain ATCC 10876 of *B. cereus* were produced on solid medium for sporulation. The spores were collected after we verified that the level of sporulation was about 95%. The suspension was collected in saline solution 0.9%, and then it was incubated at 56°C for 30 minutes in order to remove all the vegetative forms. After incubation, the spores were washed 3 times in saline solution 0.9%. The stability of the solution containing spores has been verified by plate count carried out every 2 weeks for 3 months. In the tests, we used a stabilized suspension N_{cereus} containing $6.7\times10^8\pm20\%$ spores CFU/ml equal to $8.7\leq\log N_{cereus}\leq8.9$.

Yersinia pestis

A pathogenic strain of *Y. pestis* (strain NCTC 02028), the agent of plague, was used for this experiment. The strain was grown in 5% sheep blood agar plates. After incubation at 37°C for 48 hours, a single colony was picked and streaked on a CIN agar and incubated at 37°C for 48 hours. In the tests, we used a stabilized suspension $N_{yersinia}$ containing $2.1\times10^9\pm20\%$ CFU/ml equal to $9.2\leq\log N_{yersinia}\leq9.4$.

Burkholderia mallei

A pathogenic strain of *B. mallei* (strain ATCC 23344), the agent of glanders, was used for this experiment. The strain was grown in 5% sheep blood agar plates. After incubation at 37°C for 48 hours, a single colony was picked and streaked on 5% sheep blood agar plates and incubated at 37°C for 48 hours. In the tests, we used a stabilized suspension N_{mallei} containing $4.9\times10^9\pm20\%$ CFU/ml equal to $9.6\leq\log N_{mallei}\leq9.8$.

Burkholderia pseudomallei

A pathogenic strain of *B. pseudomallei* (strain A 101-10) was used in this experiment. The strain was grown on agar Ashdown LR. After incubation at 37°C for 24 hours, a single colony was picked and streaked on agar Ashdown plate and incubated at 37°C for 24 hours. In the tests, we used a stabilized suspension $N_{pseudomallei}$ containing $3.2\times10^9\pm20\%$ CFU/ml equal to $9.4\leq\log N_{pseudomallei}\leq9.6$.

Francisella tularensis ssp. *tularensis*

A pathogenic strain of *F. tularensis* (strain SCHU S4), the agent of tularemia, was used in this experiment. The strain was grown in 5% sheep blood agar plates. After incubation at 37°C for 48 hours, a single colony was picked and streaked on a plate of agar with 9% chocolatized sheep blood, supplemented with antibiotics (CHAB-A) and incubated at 37°C for 48 hours. In the tests, we used a stabilized suspension $N_{tularensis}$ containing $2.6\times10^9\pm20\%$ CFU/ml equal to $9.3\leq\log N_{tularensis}\leq9.5$.

Brucella abortus and *Brucella melitensis*

Pathogenic strains of *B. abortus* and *B. melitensis* (B19 vaccine strain and RKI 16 M), the agents of brucellosis, were used in this experiment. The strains were separately grown in 5% sheep blood agar. After incubation at 37°C for 48 hours, a single colony was picked and streaked on a plate of brucella agar 5% and incubated at 37°C for 48 hours. In the tests, we used a stabilized suspension $N_{abortus}$ containing $2.3\times10^9\pm20\%$ CFU/ml equal to

$10.2 \leq \log N_{abortus} \leq 10.4$ and a stabilized suspension $N_{melitensis}$ containing $1.5 \times 10^9 \pm 20\%$ CFU/ml equal to $10.1 \leq \log N_{melitensis} \leq 10.3$.

Pathoster® 0.35% and Pathoster® 0.50%

Pathoster® 0.35% and Pathoster® 0.50% are cold chemical sterilants in compliance with UNI EN 14937:2009, characterized by a chemical composition based on stabilized peracetic acid. (Pathoster® sterilants are produced by Cerichem Biopharm SRL, Cerignola (FG), Italy.) Pathoster® 0.35% is a medical device registered with number 1361127/R and CND code D050101. The products contain non-ionic copolymers compatible with the oxidizing solution, consisting of a hydrophobic central part and hydrophilic lateral chains and a mixture of carboxylic acids. These chemical compounds generate a slow release of peracetic acid that allows long life of the products. The products consist of 2 components: The first one is the activator, and the second one is the stabilizer. The 2 components must be mixed before use with the ratio of 75 ml activator and 625 ml stabilizer. The result of the mixing will be the reconstituted product, ready for use. The sterilizing solution of Pathoster® 0.35% ready for use consists of 0.32% peracetic acid, 1.41% of hydrogen peroxide, aqueous buffer solution, corrosion inhibitors, non-ionic surfactants, complexing agents, and stabilizers. The sterilizing solution of Pathoster® 0.50% ready for use consists of 0.46% peracetic acid, 2.2% of hydrogen peroxide, aqueous buffer solution, corrosion inhibitors, non-ionic surfactants, complexing agents, and stabilizers. Both formulations show good compatibility with materials except for aluminum, copper and corresponding alloys, and natural gums. The activator showed an oral LD_{50} for rats of 1540 mg/kg, a dermal LD_{50} for rats of 1410 mg/kg, and an inhalation LC_{50} of 450 mg/m^3.

Stability Test of the Products

After being reconstituted, the products were kept at an ambient temperature (between 23°C and 26°C) throughout the experimental period. A quantity of 100 µl of the N suspension of each bacterium examined in this work was added to 900 µl of product, and the solution obtained was kept at room temperature for 5 minutes before proceeding to scalar dilutions in physiological solution. For *B. anthracis* and *B. cereus,* the considered dilutions were 10^{-6} and 10^{-7}, while for all the remaining studied bacteria the dilutions considered were 10^{-7} and 10^{-8}.

The above dilutions were chosen to optimize the reading of the plates. For each dilution, 3 plates of specific agar medium were used for each bacterium (Table 1), and each plate was seeded with 100 µl of solution containing bacteria and Pathoster. After seeding, the 3 plates were aerobically incubated at 37°C and the readings of colonies were made at 24, 48, and 72 hours. The test was carried out for both products. The vitality and the microbial load of each initial bacterial suspension was verified daily.

As a negative control, we used a solution containing bacteria, and, instead of the products, we used physiological saline solution at 0.9% NaCl. The same technique was used to verify the level of effectiveness after 15 minutes of contact between bacteria and Pathoster. The limit of effectiveness was the period after which the product was unable to exert a complete sterilization after contact with at least 1 of the 8 bacterial suspensions considered in this work. All experiments were repeated 3 times, and the data provided are the averages of the results.

Residual Activity

The residual activity is the effectiveness of the product after a dilution with aqueous solutions. In order to verify the residual activity of Pathoster® 0.35% and Pathoster® 0.50%, scalar dilutions of the products were performed in physiological saline solution at 0.9% NaCl. The prepared dilutions ranged from 10^{-1} to 10^{-8}; in each dilution a known amount of spores or bacteria was added. We used the same bacteria used in the experiment. We carried out a 30-minute incubation aerobically at room temperature, and subsequently each dilution was seeded in 3 plates containing a specific medium for the bacterium used. The perfect match between the number of bacteria inserted in the solution and those subsequently detected during the reading of plates was defined as "absence of residual inhibitory activity." In this case, after the first dilution of 1:10, the products had no activity on bacteria. The inactivation of the antimicrobial activity of products in the diluted solution ensures the accuracy of the contact time of our experiments.

Results

Figures 1 and 2 show the graphs relating the stability of Pathoster® 0.35% and Pathoster® 0.50% in the test at 5 minutes of contact with the bacteria used in this work. Pathoster® 0.35% maintained its sterilizing efficacy against spores of *B. anthracis* on average for 17 ± 1 days, while against spores of *B. cereus* the sporicidal activity was preserved on average for 16 ± 1 days. As far as the other bacteria, the sterilizing action persisted at least until the 26th day. Pathoster® 0.50% retained full sterilizing activity against spores of *B. anthracis* for 25 ± 1 days, while against spores of *B. cereus* it maintained its effectiveness for 24 ± 1 days. As regards the other bacteria, Pathoster® 0.50% retained its sterilizing effectiveness at least until the 35th day.

Figures 3 and 4 show the graphs relating the stability of Pathoster® 0.35% and Pathoster® 0.50% in the test at 15 minutes of contact with the bacteria used in this work. Pathoster® 0.35% retained its sterilizing efficacy against spores of *B. anthracis* for 20 ± 1 days, while against spores of *B. cereus* it maintained its effectiveness for 21 ± 1 days. Pathoster® 0.50% preserved its effectiveness against spores

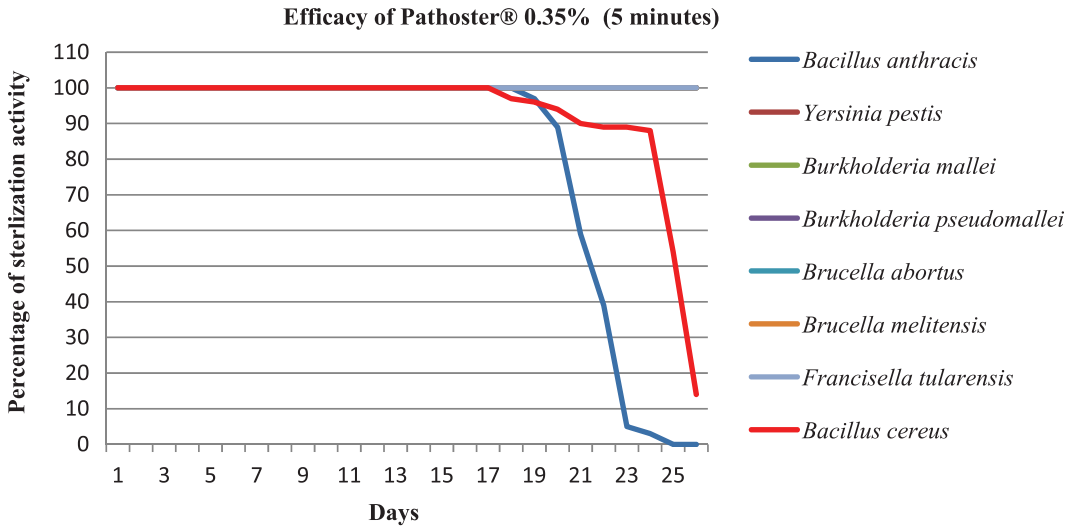

Figure 1. The Stability of Pathoster® 0.35% After Reconstitution. Efficacy against bacteria after a contact time of 5 minutes

of *B. anthracis* after a contact of 15 minutes for 27 ± 1 days, while against spores of *B. cereus* it maintained its effectiveness for 24 ± 1 days. The sterilizing activity of the 2 products against other bacteria lasted for a longer time compared to the sterilizing activity recorded against the spores of *B. anthracis* and spores of *B. cereus*.

The results of this work confirm the sporicidal and biocidal activity of peracetic acid and, in particular, the effectiveness after a short contact (5 minutes). Specifically, Pathoster® 0.35% maintained its sporicidal efficacy after reconstitution for 16 ± 1 days and Pathoster® 0.50% for 24 ± 1 days. After these periods, a rapid and progressive decay of sporicidal activity against spores of *B. anthracis* and spores of *B. cereus* was observed for both products. Pathoster® 0.35%, after 19 days from its reconstitution and when placed in contact with spores of *B. anthracis* for 5 minutes, was effective on 97% of the spores. However, on the 21st day the effectiveness is about 59%, and it completely vanished around the 25th day. As regards the action against spores of *B. cereus*, it was verified that Pathoster® 0.35%, after 18 days from its reconstitution and when placed in contact for 5 minutes with spores, was still able to inactivate 97% of the spores. The decline of its activity was slower, and on the 25th day it was still able to inactivate 54% of spores; on the 26th day it was able to inactivate 14% of spores.

The same trend was observed for Pathoster® 0.50%, which, after 27 days since its reconstitution, appeared to have sporicidal action against spores of *B. anthracis* equivalent to 99%. In the following days, the sporicidal action slowly decreased to a level of 12% after 34 days and 1%

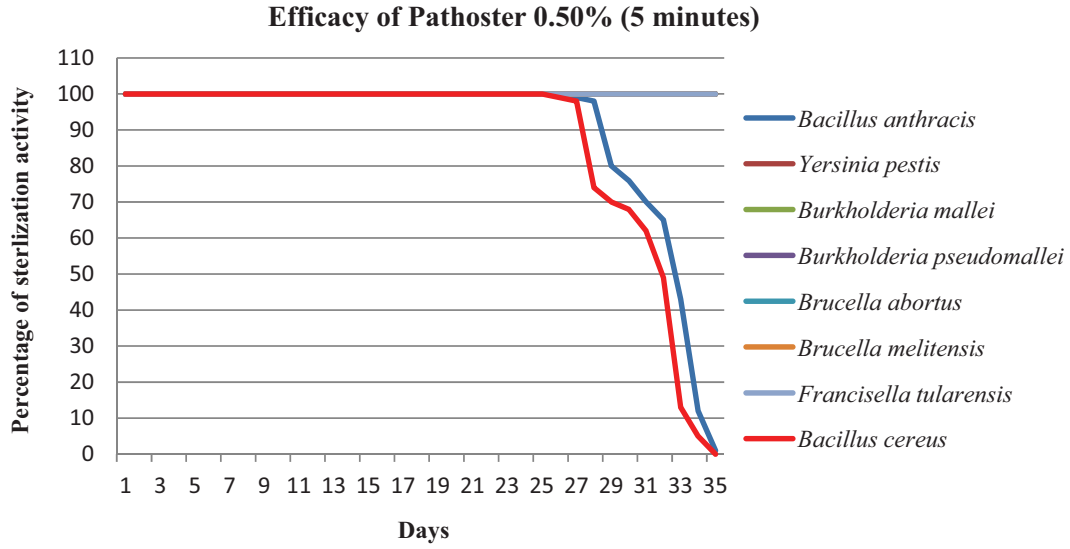

Figure 2. The Stability of Pathoster® 0.50% After Reconstitution. Efficacy against bacteria after a contact time of 5 minutes

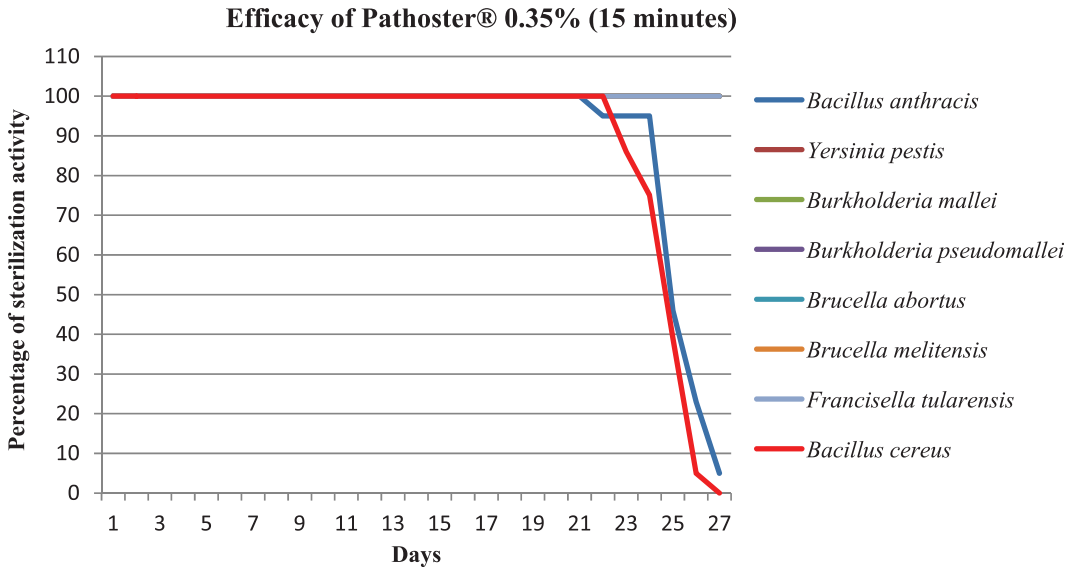

Figure 3. The Stability of Pathoster® 0.35% After Reconstitution. Efficacy against bacteria after a contact time of 15 minutes

after 35 days. Even in the case of Pathoster® 0.50%, the limit was determined by measuring the sporicidal activity against the suspension of spores of *B. cereus*; after 24 ± 1 days, it was observed that 1% (99% effectiveness of the product) of the spores survived to the contact for 5 minutes.

The data concerning the conservation of biocidal activity and sporicidal activity of the 2 products in the case of exposure to bacteria for 15 minutes indicate that Pathoster® 0.35% lost its sterilizing efficacy against spores of *B. anthracis* (survival of 5% of spores) after 22 ± 1 days, while as regards the activity on the spores of *B. cereus*, on the 23rd day there was a loss of effectiveness of 14%. Pathoster® 0.35%, with a time of contact of 5 minutes with spores of *B. anthracis* or *B. cereus*, completely lost its effectiveness after 27 ± 1 days. Pathoster® 0.50%, with a time of contact of 15 minutes, showed a slower loss of efficacy: After 35 ± 1 days from its reconstitution, it had an efficacy equal to 79% on spores of *B. anthracis* and 76% on spores of *B. cereus*. However, although the data are indicative of the real effectiveness, these data are the result of direct contact of the bacterium with the sterilant.

Discussion

Peracetic acid has excellent biocidal and sporicidal activity.[1,3,20,21] Unfortunately, aqueous solutions of peracetic acid have short-term efficacy, because degradation processes

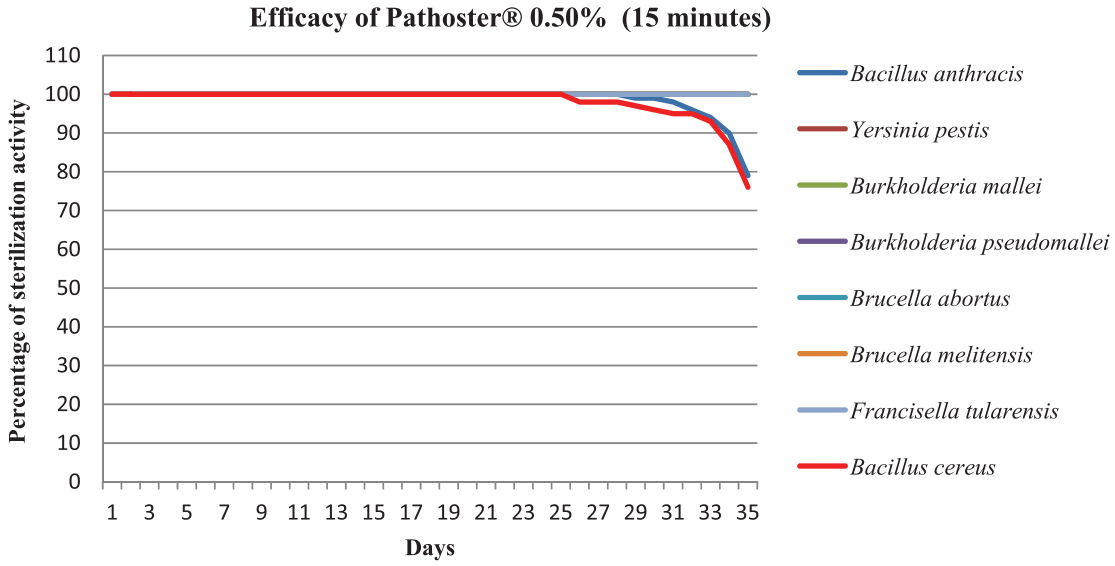

Figure 4. The Stability of Pathoster® 0.50% After Reconstitution. Efficacy against bacteria after a contact time of 15 minutes

cause a strong reduction of their activity.[1,4] Such instability is detected in the majority of products based on peracetic acid currently on the market. The rapid loss of activity is a disadvantage because, in order to be assured of the effectiveness of the treatment, operators are forced to reconstitute the product weekly.

Recently, to optimize the use of peracetic acid against spores, it has been proven that the application of germinants increases the sensitivity of bacterial spores to peracetic acid.[28] One of the most interesting characteristics of Pathoster® is related to the long time stability, determined by the addition of a stabilizer that is able to slow down the process of degradation. In particular, the products stored after reconstitution at room temperature keep their sterilizing power unchanged, after an exposure of 5 minutes, for over 2 weeks in the case of Pathoster® 0.35% and for more than 3 weeks in the case of Pathoster® 0.50%.

This article assessed the effectiveness of peracetic acid against spores of *B. anthracis* and *B. cereus* and vegetative forms of *Y. pestis*, *B. mallei*, *B. pseudomallei*, *F. tularensis*, *B. abortus,* and *B. melitensis*, confirming peracetic acid as a biocide and sporicidal agent of election against the main bacteria agents with potential bioterrorism use.[1] However, it must be emphasized that the tests conducted in this work were carried out at temperatures between 23°C and 30°C and that the sterilizing activity of peracetic acid at low concentrations and at short incubation times is less effective at higher temperatures.[29] In this regard, Kunigk and colleagues showed that at 45°C the effectiveness of peracetic acid decreases by 50% in 72 hours, while at 25°C the effectiveness decreases by 33% after 10 days.[30] In the field of biological control it is very important to know the exact period during which a sterilant is effective, because this information allows considerable cost savings and also facilitates the work of operators. Because of its characteristics, Pathoster® 0.35% could be indicated for the sterilization of closed working environments such as laboratories, hospitals, operating rooms, or other working places, as the low content of peracetic acid greatly reduces the risks connected to the residual toxicity. A potentially disruptive effect on eyes and the respiratory tract due to acetic smell has been reported in the literature.[4,31] The use of Pathoster® 0.35% is also recommended for environments in which the temperature is continuously monitored and constant throughout the year. Conversely, the product with a concentration of 0.50% peracetic acid is more suitable for outdoor use (eg, decontamination of means of transport of urban and special waste) and in the sterilization of large work environments such as contaminated slaughterhouses and floors.

Acknowledgments

This project was partially funded by Cerichem Biopharm SRL (www.cerichem.com).

References

1. Frentzel H, Menrath A, Tomuzia K, Braeunig J, Appel B. Decontamination of high-risk animal and zoonotic pathogens. *Biosecur Bioterror* 2013;11(Supp 1):102-114.
2. *Australian Veterinary Emergency Plan (AUSVETPLAN) Operational Procedures Manual: Decontamination* (Version 3.2). Canberra: ATC Animal Health Australia; 2008.
3. Sella SRBR, Guizelini BP, Ribeiro H. Validation of peracetic acid as a sporicide for sterilization of working surfaces in biological safety cabinets. *J Microbiol Infect Dis* 2012;2(3): 93-99.
4. Block SS. *Disinfection, Sterilization and Preservation.* 4th ed. Philadelphia: Lea & Febiger; 1991.
5. Fasanella A, Adone R, Hugh-Jones M. Classification and management of animal anthrax outbreaks based on the source of infection. *Ann Ist Super Sanità* 2014;50(2):192-195.
6. McGregor D, Bolt H, Cogliano V, Ritcher-Reichhelm HB. Formaldehyde and glutaraldehyde and nasal cytotoxicity: case study within the context of the 2006 IPCS Human Framework for the analysis of a cancer mode of action for humans. *Crit Rev Toxicol* 2006;36(10):821-835.
7. World Gastroenterology Organization. *WGO-OMGE and OMED Practice Guideline: Endoscope Disinfection*. 2005. http://www.srgh.ro/fisiere/dezinfectia_endoscoapelor.pdf. Accessed June 10, 2016.
8. Kunigk L, Almeida MCB. Action of peracetic acid on Escherichia coli and Staphylococcus aureus in suspension or settled on stainless steel surfaces. *Braz J Microbiol* 2001; 32(1):38-41.
9. Wutzler P, Sauerbrei A. Virucidal activity of the new disinfectant monopercitric acid. *Lett Appl Microbiol* 2004;39(2):194-198.
10. Bore E, Langsrud S. Characterization of micro-organisms isolated from dairy industry after cleaning and fogging disinfection with alkyl amine and peracetic acid. *J Appl Microbiol* 2005;98(1):96-105.
11. Dychdala GR. New hydrogen peroxide–peroxyacetic acid disinfectant. In: Proceedings of the 4th Conference Prog Chemistry; 1988;315-342.
12. Schroder W. Peracetic acid. Disinfectant for the food industry. *Brauwelt Int* 1984;115-120.
13. Fraser JAL, Godfree AF, Jones F. Use of peracetic acid in operational sewage sludge disposal to pasture. *Water Sci Technol* 1984;17:451-466.
14. Freer PC, Novy FG. On the formation, decomposition and germicidal action of benzoylacetyl and diacetyl peroxides. *Am Chem J* 1902;27:161-193.
15. Hutchings IJ, Xezones H. Comparative evaluation of the bactericidal efficiency of peracetic acid, quaternaries and chlorine containing compounds. In: Proceedings of the 49th Annual Meeting of the Society Am 1949;50-51.
16. Greenspan FP, MacKellar DG. The application of peracetic acid germicidal washes to mold control of tomatoes. *Food Technol* 1951;5:95-97.
17. Liberti L, Notarnicola M. Advanced treatment and disinfection for municipal wastewater reuse in agriculture. *Water Sci Technol* 1999;40:235-245.
18. Baldry MGC, Fraser JAL. Disinfection with peroxygens. In: Payne KR, ed, *Industrial Biocides.* New York: Wiley; 1988: 91-116.

19. Leaper S. Synergistic killing of spores of Bacillus subtilis by peracetic acid and alcohol. *J Food Technol* 1984;19:355-360.
20. Tutumi M, Imamura K, Hatano S, Watanabe T. Antimicrobial action of peracetic acid. *J Food Hyg Soc* 1973;15: 116-120.
21. Leggett MJ, Schwarz JS, Burke PA, McDonnell G, Denyer SP, Maillard JY. Resistance to and killing by the sporicidal microbicide peracetic acid. *J Antimicrob Chemother* 2015; 70(3):773-779.
22. Gehr R, Cochrane D, French M. Peracetic acid as a disinfectant for municipal wastewaters: encouraging performance results from physicochemical as well as biological effluents. In: Proceedings of the U.S. Water Environment Federation Disinfection Conference; 2002.
23. Wagner M, Brumelis D, Gehr R. Disinfection of wastewater by hydrogen peroxide or peracetic acid: development of procedures for measurement of residual disinfectant and application to a physicochemically treated municipal effluent. *Water Environ Res* 2002;74(1):33-50.
24. Colgan S, Gehr R. Disinfection. *Water Environ Technol* 2001;13:29-33.
25. Lefevre F, Audic JM, Ferrand F. Peracetic acid disinfection of secondary effluents discharged of coastal seawater. *Water Sci Technol* 1992;25:155-164.
26. Sanchez-Ruiz C, Martinez-Royano S, Tejero-Monzon I. An evaluation of the efficiency and impact of raw wastewater disinfection with peracetic acid prior to ocean discharge. *Water Sci Technol* 1995;32:159-166.
27. Yuan Z, Ni Y, van Heiningen ARP. Kinetics of peracetic acid decomposition: Part I. Spontaneous decomposition at typical pulp bleaching conditions. *Can J Chem Eng* 1997;75:37-41.
28. Celebi O, Buyuk F, Pottage T, et al. The use of germinants to potentiate the sensitivity of *Bacillus anthracis* spores to peracetic acid. *Front Microbiol* 2016;7:18.
29. Sudhaus N, Pina-Pérez MC, Martínez A, Klein G. Inactivation kinetics of spores of Bacillus cereus strains treated by a peracetic acid-based disinfectant at different concentrations and temperatures. *Foodborne Pathog Dis* 2012;9(5):442-452.
30. Kunigk LDR, Gomes FF, Vidal KP, Gomes LF, Sousa PF. The influence of temperature on the decomposition kinetics of peracetic acid in solutions. *Braz J Chem Eng* 2001;18(2):217-220.
31. Pechacek N, Osorio M, Caudill J, Peterson B. Evaluation of the toxicity data for peracetic acid in deriving occupational exposure limits: a minireview. *Toxicol Lett* 2015;233(1):45-57.

Manuscript received January 7, 2016;
accepted for publication March 29, 2016.

Address correspondence to:
Emanuele Campese
Istituto Zooprofilattico Sperimentale of Puglia and Basilicata
Anthrax Reference Centre of Italy
via Manfredonia, 20
Foggia 71121 Italy

Email: emanuele.campese@izspb.it