OLYMPUS®

Your Vision, Our Future

Acecide®

High-level Disinfectant

As the World's Leader in Endoscopy Products,
We Know What Makes an Ideal Endoscope Disinfectant

Contents

1. What Is Acecide® and Why Should You Use It?

As the worlds leader in endoscopy products, we know what it takes to make an ideal endoscope disinfectant. Acecide is the only high-level disinfectant offered by an endoscope developer and manufacturer, which guarantees the best results.

Acecide is a trademark of SARAYA CO.,LTD.

Especially designed for the sensitivity and precision of Olympus flexible endoscopes

- As precision instruments constructed with great care, endoscopes require an exceptionally gentle, yet powerful cleaning solution. Acecide was made possible by Olympus in-depth knowledge of endoscope construction and maintenance requirements.
- Although primarily composed of peracetic acid, Acecide's original formula is optimized for endoscopes.
- Optimal balance between composition, concentration, and usage temperature achieves excellent efficacy and endoscope compatibility at the same time.

Wide-range microorganism elimination

- Wide and rapid effectiveness, capable of eliminating viruses, general bacteria, acid-fast bacteria, and spores.
- Highly effective at room temperature, with no need to be heated.

Viruses	Herpes simplex virus type 1	Adenovirus type 5	Poliovirus type 3
Bacteria	Staphylococcus aureus	Pseudomonas aeruginosa	Escherichia coli
Acid-fast bacteria	M. tuberculosis	M. avium	M. kansaii
Fungi	Apergillus niger	Candida albicans	Filobasidiella neoformans

- 5-minute action time at room temperature in the OER-AW.
- Automatic preparation in the disinfectant tank of the OER-AW.
- The pH of the active solution is about 3.5 to 3.8.
- Acecide Test Strip is available for confirmation of the minimum recommended concentration.

Since Olympus introduced Acecide in 2001, it has achieved wide popularity among our users in the Japanese market, capturing a large share of the market and making a solid contribution to the achievement of high-quality reprocessing.

Optimal solution for patients, staff, and endoscopes made possible by Olympus

Scope-Friendly

- Compatibility with Olympus flexible endoscopes supported by Olympus through validation tests.
- No minimal Endoscope deterioration in reprocessing thanks to the dedicated formula.

Environmentally Friendly

- Less water for preparation than single use.
- Less wastewater discharge.
- Fewer bottles to dispose of.

Simple Preparation

- Easy setup with dedicated bottle design.
- Automatic feeding of disinfectant into the OER-AW's tank.
- Lower risk of direct contact with the disinfectant during preparation.

And there are many more advantages...

- The reusable design helps streamline daily procedures in your facility.
- The minimum recommended concentration (MRC) of Acecide can be confirmed by Acecide Test Strip.

2. Peracetic Acid Overview

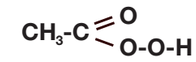
User friendly

While there are reports of sensitization in humans caused by aldehyde chemicals, there have been no reports of peracetic acid causing allergies or sensitization,[1] when used as directed and wearing appropriate protective equipment.

- **Peracetic acid** → Peracetic Acid does not contain an aldehyde material.
- **Glutaraldehyde**
- **Ortho-phthaladehyde** → Contains an aldehyde material.

Environmentally friendly

Peracetic solution decomposes rapidly into water and oxygen.



$$CH_3COOOH + H_2O \rightleftharpoons CH_3COOH + H_2O_2$$

Peracetic acid + Water — Acetic acid + Hydrogen peroxide

When peracetic acid breaks down, hydrogen peroxide and acetic acid are produced. Hydrogen peroxide, in turn, is easily decomposed to oxygen and water as practically a not toxic material.[2, 3]

Non-bacteria resistant

No bacteria resistant to peracetic acid have been reported. It is generally believed that the resistant bacteria are unlikely to be generated because peracetic acid has multiple action mechanisms. Several different action mechanisms have been suggested for peracetic acid:[3]

- degeneration of cellular proteins and inhibition of cell transportation,
- inactivation of the enzymes essential for metabolism, and
- destruction of cellular membrane and its permeability.

Non-protein coagulation

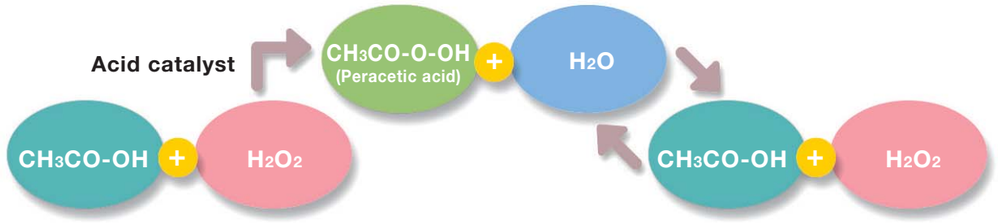
Peracetic acid and hydrogen peroxide, two components of Acecide, oxidize and break down organic matter which eases their removal. Unlike glutaralydehyde, it does not cross-link with proteins and as a result, it does not cause blood coagulation.[4, 5]

Source:
[1] Malchesky, P.S., Disinfection, Sterilization, and Preservation. 5th ed. (ed. By Block, S.S.), Philadelphia: Lippincott Williams & Wilkins, p.979-996, 2000.
[2] Dychdala, G. R. Proc 4th Conf. Chem. Disinfection, New York State University, Binghamton, NY, pp.315-342, 1988
[3] Block, S.S., Disinfection, Sterilization, and Preservation 4th Ed, Edition by S.S Block, Lea & Febiger,
[4] Tucker, R.C. et al., ASAIOJ 1996; 42: 306-313.
[5] Furuta T., The Japanese Journal of Medical Instrumentation 2000; 70: 10; 529-530.

1) Chemistry of peracetic acid

Peracetic acid has a structure in which one hydrogen atom in hydrogen peroxide is replaced by an acetyl group and, therefore, it exhibits characteristics of an acid, a peroxide as well as an alcohol.[6] Peracetic acid is produced when hydrogen peroxide and acetic acid are mixed and is present as a mixture in equilibrium with these compounds. It is degraded easily into hydrogen peroxide and acetic acid by dilution, heating, and so on. Hydrogen peroxide is degraded easily into oxygen and water through heating or reaction with organic substances, etc. These degradation products are said to be practically non-toxic.[7, 8]

Synthesis of peracetic acid by acetylation of hydrogen peroxide



2) Antimicrobial effect of peracetic acid

Although peracetic acid is derived from hydrogen peroxide and acetic acid, it has more potent antimicrobial activity than hydrogen peroxide and shows wide antimicrobial spectrum. It has been shown to be effective against Gram-negative bacteria, Gram-positive bacteria, yeast and molds, viruses and spore-forming bacteria at fairly low concentrations.[9-16,19] It exhibits sporicidal activity even at a very low concentration and its activity persists in the presence of organic substances. Optimum performance is achieved at low pH. At Alkaline pH, a higher concentration is required to obtain the same activity.

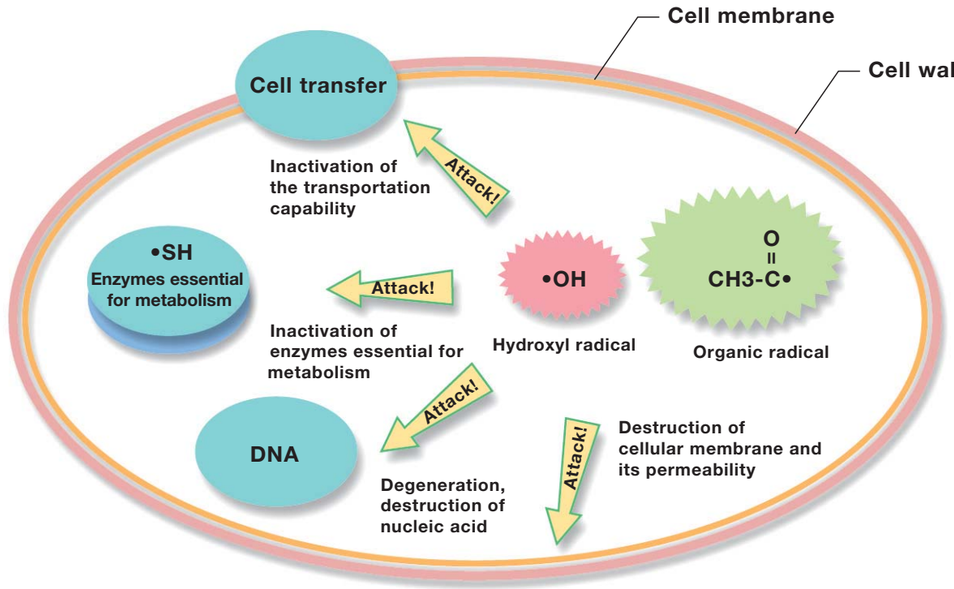
"Excellent disinfecting action and cold sterilizing activity" of peracetic acid has been reported by Freer and Novy in 1902.[17] Peracetic acid is one of most potent antimicrobial agents and its bactericidal activity belongs to the high level among various disinfectants reported. It exhibits excellent activity in almost all the comparative studies conducted by various researchers. Peracetic acid is also effective on biofilms in comparison to the general disinfectants used in practice.[18]

3) Mechanism of action

The exact mechanism of action of Peracetic acid is not clearly known. Several theories have been suggested by various researchers. In summary, destruction of microbial cells by peracetic acid can be classified into three different mechanisms:

(1) Degeneration of cellular proteins and inhibition of cell transportation,
(2) Inactivation of the enzymes essential for metabolism, and
(3) Destruction of cellular membrane and its permeability.

Destruction of microbial cells are triggered by the production of activated radicals inside bacterial cell bodies



4) Studies

Greenspan et al. considered that the mechanism of antimicrobial activity of peracetic acid differed slightly from hydrogen peroxide because it is not affected by catalase which degrades hydrogen peroxide.[20]

Marquis et al. have reported that the bactericidal effect of peracetic acid is attributed to radical formation and thus, not associated with hydrogen peroxide since reduced types of copper, iron and cobalt show protective effect against bactericidal activity of peracetic acid whereas their oxidized types as well as Mn+, K+ and chelating agents have no effect.[21]

Davis et al. have proposed that peracetic acid would eradicate bacteria by breaking sulfhydryl (-SH) and disulfide (S-S) bonds in proteins and enzymes by oxidation.[22]

Baldry and Fraser state that protozoicidal and sporicidal activities of peracetic acid are associated with protein degeneration and, in proteins, enzymes and other metabolites, oxidation of reactive mercapto groups and disulfide bonds and reactions with double bonds are probably taking place.[23]

Pavlova and Kulikovski have shown that bactericidal and sporicidal activities are attributed to destruction of permeability in bacterial cell bodies. It has been described that peracetic acid reacts with proteins and, thus, disrupts chemical osmotic function and transportation in the lipoprotein cell membrane through disturbance and destruction of bacterial cell walls.[24,25]

Clapp et al. (1994), in a recent electron paramagnetic resonance (EPR, or electron spin resonance) study by the spin trapping method, showed that 5,5-dimethyl-1-pyrroline N-oxide (DMPO) used as a spin trap and two antioxidants (vitamin C and Trolox C) inhibited bactericidal activity and, thus, confirmed that the hydroxyl radical is a bactericidal chemical species and have shown that this hydroxyl radical is produced by the reaction between bacteria and peracetic acid. [26] They also showed that an active bactericidal radical was produced within the microbial bodies. Bacteria cultured in an iron-rich medium showed increased susceptibility to bactericidal activity but addition of iron ions to a mixture of peracetic acid and bacteria had no effect on the bactericidal activity. The effect of iron chelating agent and addition of a haem protein inhibitor indicated involvement of iron and haem protein, in particular, in the bactericidal property of peracetic acid.

Maillard et al. examined the mechanism of virus inactivation by peracetic acid using bacteriophages as the model and revealed that peracetic acid induced changes in the structure (both in capsid and tail), proteins and nucleic acids in F116 phage.[27]

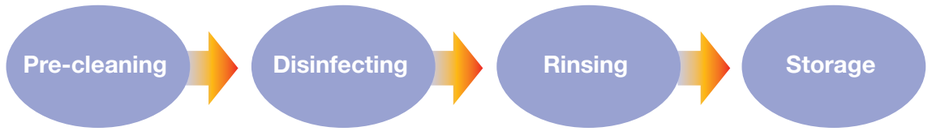
Malchesky has summarized the above mechanisms of action of peracetic acid in his general review.[28]

Source:

6 Ogata, Y., Chemistry of Organic Peroxides, p. 100, Nankodo, Tokyo, 1970. (In Japanese)
7 Dychdala, G. R. Proc 4th Conf. Chem. Disinfection, New York State University, Binghamton, NY, pp. 315-342, 1988.
8 Block, S.S., Disinfection, Sterilization, and Preservation 4th Ed, Edition by S.S Block, Lea & Febiger, Philadelphia, pp. 172-179, 1991.
9 Baldry, M.G. C., The bactericidal, fungicidal, and sporicidal properities of hydrogen peroxide and peraceti acid J.Appl. Bacteriol. 1983;4:417-423.
10 Eggensberger, H., Zentralbl. Bakteriol. Mikrobiol. Hyg. [B], 1979;168:517-524.
11 Baldry, M, G. C. and Fraser, J.A. L., Industrial Biocides. Edited by K. R. Payne, John Wiley & Sons, NY. pp. 91-116, 1998.
12 Sprossig, M., Resistance of Microorganisms to Disinfectants: Second International Symposium. Edited by W. B. Kedzia, Warsaw, Polish Academy of Sciences, pp.89-91, 1975.
13 Schroeder, W., Brauwelt Int. 1984;1:115-120.
14 Block, S. S., Proc. 3rd Conf.Prong. Chem. Disinfection, New York State University, Binghamton, NY,pp. 1-28, 1986.
15 Roshner, D., Technical Bulletin, Hankel Cor-poration, p. 22 , 1987.
16 Dychdala, G. R., Disinfection , Sterilization and Preservation , 3rd ed., edited by S. S. Block, Lea and Febiger, Philadelphia, pp.157-182, 1988.
17 Cords, B. R. and Dychdala, G. R., Antimicrobials in Foods. 2nd ED., (Ed. By Davidson, P. M. and Branten, A. L.), Marcel Dekker, pp. 469-537, 1993.
18 Holah, J. T. et al., Lett. Appl. Microbiol. 1990; 11: 225-259.
19 Lynam, P. A. et al., J. Hosp. Infec 1995; 30:237-239.
20 Greenspan, F. P. et al., Proc. 42nd Ann. Mtg. Chem. Dec. 5-7, CMA, Washington, DC. Mfctrs. Assoc., pp. 59-64, 1955.
21 Marquis, R. E. et al., J. Ind. Microbiol. 1995; 15 (6): 486-492.
22 Davis, B. D. et al., Microbiology Including Immunology and Molecular Genetics, 3rd ED. Harper and Row Publishers, Inc., London, pp.1269-1270.
23 Badry, M. G. C. and Fraser, J. A. L., Industrial Biocide. Edited by K. R. Payne, John Wiley & Sons, NY, pp. 91-116, 1988.
24 Pavlova, I. B. and Kulikovski, A. V., Zh. Mikrobiol. 1978 1:37-41.
25 Fraser , J. A., Specialty CHmeicals 1987;7(3): 178-186.
26 Clapp, P . A. al., Free Rad, Res. 1994;21(3): 147-167.
27 Maillard, J. Y. et al., Sci. Prog. 1997;80:287-315.
28 Malchesky, P. S., Disinfection, Sterilization, and Preservation. 5th ed. (ed. By Block, S. S.), Philadelphia; Lippincott Williams & Wilkins, pp. 979-996, 2000.

3. Reprocessing Guide

Required steps for reprocessing using Acecide active solution



1. Safety

- Wear protective glasses, gloves and mask to avoid contact and inhalation of disinfectant and infectious material.

2. Pre-cleaning

- Remove the contaminants with the Olympus cleaning brush before disinfection.

3. Preparing the active solution

- Prepare by mixing Reagent One, Reagent Two and purified water to make Acecide active solution.

4. Checking the concentration

- Check the concentration of Acecide active solution before every cycle.

5. Applicable devices

- Endoscopes, lensed equipment, obstetric and urologic instruments are applicable. For more information, please contact the Olympus office.

6. Disinfecting

- Soak for 5 minutes or more. If spore elimination is required, soak for 10 minutes.
- Select the programs of the OER-AW based on your requirement.

7. Rinsing

- Rinse the instruments so that no disinfectant remains after the disinfecting cycle has finished.

8. Storage

- Dry the instruments to prevent recontamination.

Note: Please refer to the instruction manual provided with this product for complete inspection criteria, warnings, cautions and instructions for use.

4. FAQs

Q.1 Are Olympus endoscopes compatible to be disinfected with the combination of Acecide and the OER-AW?

A.1 Yes. We confirmed the compatibility of Olympus flexible Endoscopes for reprocessing with the combination of Acecide and the OER-AW.

Q.2 What scope types are compatible?

A.2 Acecide is compatible with Olympus gastrointestinal endoscopes, bronchoscopes, nasopharyngolaryngoscopes and cholangioscopes. For detailed information on model name, please contact your local Olympus sales representative.

Q.3 Are non-Olympus endoscopes compatible with Acecide and OER-AW?

A.3 No. Compatibility cannot be guaranteed because we do not have detailed information regarding the construction and specifications of non-Olympus endoscopes.

Q.4 Is it possible to reprocess re-usable endotherapy accessories with Acecide?

A.4 No. Re-usable Endotherapy accessories should be reprocessed separately according to the sterilization protocol specified by the Olympus operating manual/instruction for use and your local guidelines.

Q.5 Can we use Acecide for natural rubber and crude rubber products?

A.5 No. Acecide should be avoided because of the possibility of deterioration.

Q.6 Can we use Acecide for iron, copper and brass?

A.6 No. Iron, copper, brass, zinc steel sheets and carbon steel are incompatible due to the potential for corrosion.

Q.7 Is there any report about health concerns associated with the use of Acecide?

A.7 No. When used as directed, and wearing appropriate protective equipment, at this stage, there have been no reports of allergies or sensitivity associated with the use of peracetic acid including Acecide. [29]

Q.8 Is there any mutagenic substance in Acecide?

A.8 No. Acecide is not designated as a mutagen. [30]

Q.9 How can we check the minimum effective concentration?

A.9 The minimum effective concentration is to be confirmed by monitoring the Acecide Test Strip. Acecide can be reused until the active solution's concentration is equal to or greater than the minimum effective concentration of 0.2% (please see "4. Stability").

Q.10 Is there any specific condition of handling Acecide?

A.10 Yes. As much as possible please handle, transport and store Acecide between 0°C and 25°C, and according to specific conditions in the MSDS.

Q.11 How long can we store Acecide?

A.11 The shelf life of Acecide is one year after the manufacturing date.

Source:

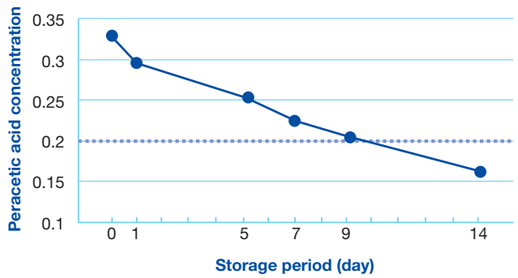
[29] Furuta T., Hospital Supply 2001; 5(2): 68-73.
[30] Malchesky, P.S., Disinfection, Sterilization, and Preservation. 5th ed (ed. By Block, S.S.), Philadelphia: Lippincott Williams & Wilkins, pp. 979-996, 2000.

5. Stability

1) Re-use cycle

Acecide can be reused until the active solutions concentration is equal to or greater than the minimum effective concentration of 0.2%. Acecide is effective after preparation for one week maximum (at room temperature). After opening, Acecide shows degradation equivalent to three cycles of use per day even if it is not used. Acecide active solution can be checked for the minimum recommended concentration by monitoring the coloration of Acecide Test Strip.

Figure 1: Stability of Acecide active solution

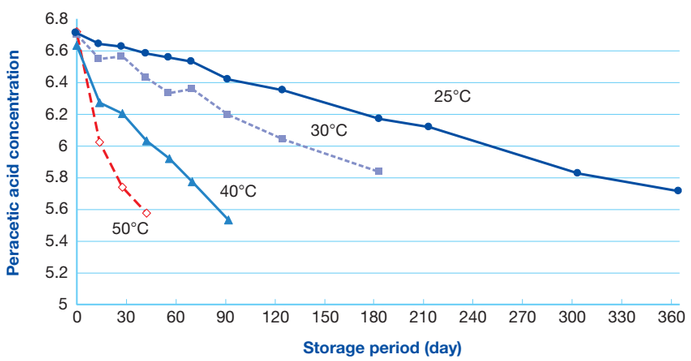


Note:
Always check the concentration of Acecide active solution before every reprocessing. Confirm efficacy with Acecide Test Strip. Be sure to replace Acecide active solution before efficacy is lost.

2) Temperature influence

Degradation of Acecide Reagent One and the active solution is accelerated at high temperatures (see Figure 2). The recommended storage temperature is < 25°C.

Figure 2: Effect of storage temperature on stability



3) Test Strip

Acecide Test Strip is used to confirm the active solution's concentration is equal to or greater than the minimum recommended concentration of 0.2%.

4) Application for Test Strip

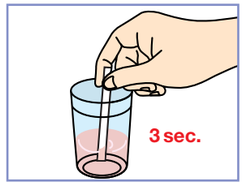
1

Tube is taken out of refrigerator and allowed to reach room temperature (10-15 minutes).

2

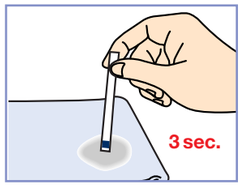
One strip is taken from the tube and tube is immediately closed afterwards.

3



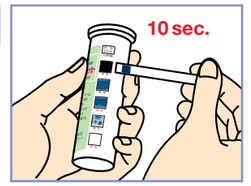
Reaction zone of test strip is soaked in active solution for 3 seconds

4



Test strip is immediately retrieved from Acecide active solution and excess solution is removed by placing strip on an absorbent paper for 3 seconds.

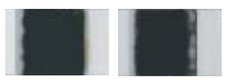
5



After 7 seconds, visual comparison of the test strip's reaction zone is made against the evaluation criteria shown on the tube.

5) Evaluation criteria

Pass



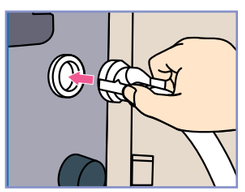
① Entire reaction zone is black to dark blue in color.
② Area 1 mm from the edge is white or partly white, but all other areas of the reaction zone are black to dark blue.

Fail

Any white spots in any area (excluding 1mm from the edge).

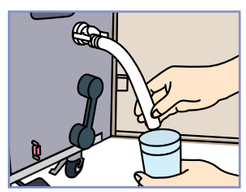
6) Sample collection

1



Open the front door of the OER-AW.
Attach the disinfectant removal tube.

2



Take a sample of disinfectant in a cup.

Note: Please refer to the instruction manual provided with this product for complete inspection criteria, warnings, cautions and instructions for use.

6. Chemical Efficacy

In vitro studies

(1) Bactericidal activity on various bacteria

Acecide active solution containing peracetic acid at a concentration (0.18%) below the minimum recommended concentration (MRC) eradicated various common bacteria including Gram-positive bacteria (excluding acid-fast bacteria) and Gram-negative bacteria within 1 minute and spores within 2.5 minutes. Acecide active solution and 2.0 w/v% glutaraldehyde solution both eradicated all vegetative bacteria within 1 minute. However, the effects of these solutions differed clearly against *B. subtilis* (spore). Bacterial growth was observed even after 10 minutes with 2.0 w/v% glutaraldehyde solution whereas Acecide containing 0.18% peracetic acid eradicated them in 2.5 minutes. It was concluded that Acecide active solution at actual use concentration exhibited bactericidal activity against common bacteria including spores equivalent to or greater than glutaraldehyde preparation. (Table 1)

Method

i) Acecide active solutions were prepared by mixing 1 mL each of Solution 1 and Solution 2 and adding sterilized purified water to make 40, 30 or 20 mL (0.18%, 0.24% and 0.35% of peracetic acid, respectively). Glutaraldehyde solution used as the positive control was prepared as instructed in the directions for use (2.0 w/v% of glutaraldehyde).

ii) 1.8 mL of each solution prepared in i) was mixed with 0.2 mL of each test microbial suspension, left to stand at room temperature and used as the test solution. Blank solutions were prepared with 1.8 mL of sterilized physiological saline solution instead of the disinfectant solutions and 0.2 mL of the respective test microbial suspensions, and were tested similarly.

iii) After completion of exposure for a designated time (1 minute, 2.5 minutes, 5 minutes or 10 minutes), Acecide active solution was inactivated by the addition of 4.5 mL of 1.0 w/v% sodium thiosulfate and 4.5 mL of 1.0 w/v% catalase to each mL of the test solution. Glutaraldehyde solution was inactivated by the addition of 9 mL of 0.5 w/v% glycine to each mL of the test solution. As the control, 9 mL of sterile physiological saline was added instead of the neutralizer to 1 mL of the test microbial suspension diluted 10-fold with sterile physiological saline solution.

iv) After inactivation, 1 mL of the test solution was added to 9 mL of SCDLP liquid medium. 1 mL of this 10-fold diluted solution was added to 9 mL of SCDLP liquid medium, incubated at 35°C for 48 hours and turbidity of the SCDLP liquid medium was examined. The growth of the test microorganism was judged positive (+) if turbidity was observed or negative (–) if turbidity was absent.

v) Evaluation method: In the above test in which a fixed amount of the respective bacteria, about 10^8 CFU/mL (or 10^8 spores/mL), was inoculated, the time (in minutes) taken to arrest bacterial growth was evaluated.

Table 1: Result of the bactericidal activity study of Acecide active solution against various bacteria

Test microorganism	Exposure time	Acecide active solution (Peracetic acid)			Glutaraldehyde
		0.18%*	0.24%*	0.35%*	2.0w/v%
Staphylococcus aureus ATCC6538P	1 minute	–	–	–	–
MRSA (oxacillin: 128 H/mL, clinical isolate)	1 minute	–	–	–	–
MRSA (oxacillin: 4 μ g/mL, clinical isolate)	1 minute	–	–	–	–
Staphylococcus epidermidis ATCC12228	1 minute	–	–	–	–
Staphylococcus hominis ATCC27844	1 minute	–	–	–	–
Enterococcus faecalis ATCC19433	1 minute	–	–	–	–
Escherichia coli ATCC25922	1 minute	–	–	–	–
Enterobacter cloacae ATCC13407	1 minute	–	–	–	–
Klebsiella planticola IFO3317	1 minute	–	–	–	–
Serratia marcescens ATCC13880	1 minute	–	–	–	–
Salmonella enteritidis ATCC13076	1 minute	–	–	–	–
Proteus vulgaris ATCC13315	1 minute	–	–	–	–
Pseudomonas aeruginosa ATCC9027	1 minute	–	–	–	–
Burkholderia cepacia ATCC25416	1 minute	–	–	–	–
Bacillus subtilis ATCC6633 (Spore type)	1 minute	+	–	–	+
	2 minutes	–	–	–	+
	5 minutes	–	–	–	+
	10 minutes	–	–	–	+

+ : shows positive bacterial growth.
– : shows negative bacterial growth.
* Working solutions were prepared by Acecide active solution one 40, 30 and 20 fold, respectively, and the concentrations were calculated from the concentration of peracetic acid in Solution 1 and specific gravities of Solution 1 and Solution 2.

(2) Bactericidal activity on various acid-fast bacteria

Acecide active solution containing peracetic acid at a concentration (0.18%) below the minimum recommended concentration (MRC) eradicated various acid-fast bacteria (*Mycobacterium tuberculosis* H37Rv, *M. avium* ATCC 25291, *M. intracellulare* ATCC 13950 and *M. kansasii* ATCC 12478) within 1 minute. The bactericidal activities of Acecide active solution of the respective concentrations did not differ from that of 2.0 w/v% glutaraldehyde solution. It was concluded that the Acecide active solution at actual use concentration exhibited bactericidal activity equivalent to glutaraldehyde solution. (Table 2)

Method

i) Acecide active solutions were prepared by mixing 1 mL each of Solution 1 and Solution 2 and adding sterilized water to make 40, 30 or 20 mL (0.18%, 0.24% and 0.35% of peracetic acid, respectively). Glutaraldehyde solution used as the control was prepared as instructed in the directions for use (2.0 w/v% of glutaraldehyde).

ii) 1.8 mL of each solution prepared in i) was mixed quickly with 0.2 mL of each test microbial suspension, left to stand at room temperature and used as the test solution. Blank solutions were prepared with 1.8 mL of stelile physiological saline solution instead of the disinfectant solutions and 0.2 mL of the respective test microbial suspensions, and were tested similarly.

iii) After completion of exposure, peracetic acid solution was inactivated by the addition of 4.5 mL of 1.0 w/v% sodium thiosulfate and 4.5 mL of 1.0 w/v% catalase. Glutaraldehyde solution was inactivated by the addition of 9 mL of 0.5 w/v% glycine. As the control, 9 mL of sterile saline was added instead of the neutralizer to 1 mL of the test microbial suspension diluted 10 fold with stelile physiological saline solution.

iv) After inactivation, 1 mL of the test solution was added to 9 mL of SCDLP liquid medium. 1 mL of this 10-fold diluted solution was added to 9 mL of Middlebrook 7H9 broth, incubated at 35ºC for 2 weeks and turbidity of the Middlebrook 7H9 broth was examined. The growth of the test microorganism was judged positive (+) if turbidity was observed or negative (–) if turbidity was absent.

v) Evaluation method: In the above test in which a fixed amount of the respective acid-fast bacteria, about 10^8 CFU/mL, was inoculated, the time (in minutes) taken to arrest bacterial growth was evaluated.

Table 2: Result of the bactericidal activity study of Acecide active solution against various acid-fast bacteria

Test microorganism	Exposure time	Acecide active solution (Peracetic acid)			Glutaraldehyde
		0.18%*	0.24%*	0.35%*	2.0w/v%
Candida albicans IFO1594	1 minute	–	–	–	–
Cryptococcus neoformans TIMM0354	1 minute	–	–	–	–
Trichophyton mentagrophytes TIMM1189	1 minute	–	–	–	–
Aspergillus niger IFO6341	1 minute	+	–	–	–
	2.5 minutes	–	–	–	–

+ : shows positive bacterial growth.
– : shows negative bacterial growth.
* Working solutions were prepared by Acecide active solution one 40, 30 and 20 fold, respectively, and the concentrations were calculated from the concentration of peracetic acid in Solution 1 and specific gravities of Solution 1 and Solution 2.

(3) Bactericidal activity on various fungi

Acecide active solution containing peracetic acid at a concentration (0.18%) below the minimum recommended concentration (MRC) eradicated *Candida albicans* IFO 1594, *Cryptococcus neoformans* TIMM 0354 and *Trichophyton mentagrophytes* TIMM 1189 within 1 minute and *Aspergillus niger* IFO 6341 within 2.5 minutes. Apart from the bactericidal activity of 0.18% peracetic acid solution against *A. niger*, a diluted Acecide and 2.0 w/v% glutaraldehyde solution did not differ in bactericidal activity and the solutions eradicated all test fungi within 1 minute. *A. niger* was eradicated in 2.5 minutes with 0.18% peracetic acid solution, but the bactericidal activities of 0.24 and 0.35% solutions were comparable to that of 2.0 w/v% glutaraldehyde solution. It was concluded that Acecide active solution at actual use concentration exhibited bactericidal activity equivalent to the glutaraldehyde solution. (Table 3)

Method

i) Acecide active solutions were prepared by mixing 1 mL each of Solution 1 and Solution 2 and adding sterilized water to make 40, 30 or 20 mL (0.18%, 0.24% and 0.35% of peracetic acid, respectively). Glutaraldehyde solution used as the control was prepared as instructed in the directions for use (2.0 w/v% of glutaraldehyde).

ii) 1.8 mL of each solution prepared in i) was mixed quickly with 0.2 mL of each test microbial suspension, left to stand at room temperature and used as the test solution. Blank solutions were prepared with 1.8 mL of sterile physiological saline solution instead of the disinfectant solutions and 0.2 mL of the respective test microbial suspensions, and were tested similarly.

iii) After completion of exposure, peracetic acid solution was inactivated by the addition of 4.5 mL of 1.0 w/v% sodium thiosulfate and 4.5 mL of 1.0 w/v% catalase. Glutaraldehyde solution was inactivated by the addition of 9 mL of 0.5 w/v% glycine. As the control, 9 mL of sterile physiological saline was added instead of the neutralizer to 1 mL of the test microbial suspension diluted 10 fold with sterile physiological saline solution.

iv) After inactivation, 1 mL of the test solution was added to 9 mL of GPLP liquid medium. 1 mL of this 10-fold diluted solution was added to 9 mL of GPLP liquid medium, incubated at 35ºC for 72 hours for *Candida albicans* and *Cryptococcus neoformans* and at 30ºC for 7 days for *Aspergillus niger* and *Trichophyton mentagrophytes* and turbidity of the GPLP liquid medium was examined. The growth of the test microorganism was judged positive (+) if turbidity observed or negative (–) if turbidity was absent.

v) Evaluation method: In the above test in which a fixed amount of the respective fungi, 10^7 to 10^8 spores/mL, was inoculated, time (in minutes) taken to arrest bacterial growth was evaluated.

Table 3: Result of the bactericidal activity study of Acecide active solution against various fungi

Test microorganism	Exposure time	Acecide active solution (Peracetic acid)			Glutaraldehyde
		0.18%*	0.24%*	0.35%*	2.0w/v%
Candida albicans IFO1594	1 minute	–	–	–	–
Cryptococcus neoformans TIMM0354	1 minute	–	–	–	–
Trichophyton mentagrophytes TIMM1189	1 minute	–	–	–	–
Aspergillus niger IFO6341	1 minute	+	–	–	–
	2.5 minutes	–	–	–	–

+ : shows positive bacterial growth.
– : shows negative bacterial growth.
* Working solutions were prepared by Acecide active solution one 40, 30 and 20 fold, respectively, and the concentrations were calculated from the concentration of peracetic acid in Solution 1 and specific gravities of Solution 1 and Solution 2.

(4) Inactivation of various viruses

Acecide active solution inactivated herpes simplex virus type 1, which is a DNA virus with envelope, adenovirus type 5 without envelope, and poliovirus type 3, which is a RNA virus without envelope.
Acecide active solution containing peracetic acid at a concentration (0.18%) below the minimum recommended concentration (MRC) inactivated herpes simplex virus type 1 and adenovirus type 5 within 2.5 minutes. With poliovirus type 3, it took 10 minutes for 0.18% Acecide active solution to lower the concentration below the detection limit whereas 0.24 and 0.35% Acecide active solution inactivated the virus within 5 minutes similarly to the glutaraldehyde solution. (Table 4)

Method

i) Viruses and cells: Herpes simplex virus type 1 and poliovirus type 3 were proliferated using Vero cells while adenovirus type 5 was proliferated using HEp-2 cells and virus infectivity titers were also measured using the same cells. The infectivity titers of the viruses used were 1.0×10^5 TCID50/25 µL, 1.8×10^5 $TCID_{50}$/25 µL and 5.6×10^4 $TCID_{50}$/25 µL, respectively, for herpes simplex virus, adenovirus and poliovirus.

ii) Acecide active solutions were prepared by mixing 1 mL each of Solution 1 and Solution 2 and adding 18, 13 or 8 mL of sterile purified water (0.36%, 0.48% and 0.70% of peracetic acid, respectively). For glutaraldehyde solution used as the positive control, a double-strength solution was prepared according to the procedure instructed in the directions for use (4.0 w/v% of glutaraldehyde).

iii) 100 µL of each solution prepared in ii) was mixed with 100 µL of each test virus suspension (the final concentrations were 0.18, 0.24 and 0.35% of peracetic acid and 2.0 w/v% of glutaraldehyde) and allowed to act for a designated time (2.5, 5 or 10 minutes).

iv) 2 µL of the reaction solution in iii) was mixed with 18 µL of an inactivating agent (Acecide: 0.5% sodium thiosulfate pentahydrate +0.5% catalase, glutaraldehyde: 0.5% glycine), and 2 mL of 2% FBS-added Eagle MEM was added to dilute 1000 fold. However, the 1000-fold diluted solutions were ultrafiltered due to cytotoxicity observed in this 1000-fold diluted solution of glutaraldehyde containing adenovirus after the operation.

v) A series of dilutions were prepared from each 1000-fold diluted solution prepared in iv) by a common ratio of 10 (100.5 for adenovirus), inoculated to cultured cells in 96-well microplates and incubated at 37°C in the presence of 5% CO_2 (for 3 days with adenovirus, 5 days with herpes simplex virus and 7 days with poliovirus).

vi) Cytopathic effect (CPE) was observed and virus infectivity titer was determined.

vii) As a negative control, physiological saline was used instead of the preparation, and tested similarly by exposure for 10 minutes.

viii) Evaluation method: The infectivity titer, TCID50, was calculated by the Behrens-Karber method using the result of CPE rating. The effect was evaluated basically in comparison to the infectivity titer of the negative control and an infectivity titer within 30 minutes was regarded as deviations.

Table 4: Result of the inactivation study of Acecide active solution on various viruses

Test microorganism	Exposure time	Acecide active solution (Peracetic acid)			Glutaraldehyde
		0.18%*	0.24%*	0.35%*	2.0w/v%
Herpes simplex virus type 1	2.5 minutes	$<5.6\times10^2$	$<5.6\times10^2$	$<5.6\times10^2$	$<5.6\times10^2$
Adenovirus type 5	2.5 minutes	$<7.5\times10^2$	$<7.5\times10^2$	$<7.5\times10^2$	$<7.5\times10^2$
Poliovirus type 3	2.5 minutes	1.0×10^3	1.8×10^3	1.0×10^3	$<5.6\times10^2$
	5 minutes	1.0×10^3	$<5.6\times10^2$	$<5.6\times10^2$	$<5.6\times10^2$
	10 minutes	$<5.6\times10^2$	$<5.6\times10^2$	$<5.6\times10^2$	$<5.6\times10^2$

* Working solutions were prepared by Acecide active solution one 40, 30 and 20 fold, respectively, and the concentrations were calculated from the concentration of peracetic acid in Solution 1 and specific gravities of Solution 1 and Solution 2.
* Unit values is TCID50/25 µL

(5) Bactericidal effect of Peracetic acid preparation on various microorganisms [31)]

In order to examine suitability of Peracetic acid preparation (SRY-PA preparation*) to the field of medical device reprocessing, its effect on inactivating pathogenic bacteria including MRSA and acid-fast bacteria, spore-forming bacteria, and fungi was examined in comparison to the glutaraldehyde solution.
0.2% peracetic acid solution (PA) and 2.0% glutaraldehyde solution (GA) eradicated pathogenic bacteria including MRSA within 15 seconds. Spores of *Bacillus subtilis* were eradicated within 1 minute by 0.2% PA and within 2.5 minutes by 2.0% GA. (Table 5) 0.2% PA eradicated all acid-fast bacteria tested within 1 minute but the effect of 2.0% GA differed depending on the strains. (Table 6) Fungi were eradicated within 5 minutes both by 0.2% PA and 2.0% GA. (Table 7) Poliovirus (7.6 log TCID50/0.2 mL) was not inactivated in 2.5 minutes with 0.2% PA or 2.0% GA but inactivation was achieved in 5 minutes. Adenovirus (5.5 log TCID50/0.2 mL) and herpes simplex virus (5.0 log TCID50/0.2 mL) were inactivated within 2.5 minutes both by 0.2% PA and 2.0% GA. (Table 8)
0.2% PA exhibited bactericidal effect equivalent or superior to 2.0% GA suggesting possible shortening of the exposure time. These results showed that the product could possibly be used to reprocess medical devices, particularly endoscopes.

* Code name of Acecide 6% disinfection solution under development.

Table 5: Bactericidal activities against various bacteria

	Acecide active solution				2.0% glutaraldehyde solution				Control
	15 seconds	30 seconds	1 minute	2.5 minutes	15 seconds	30 seconds	1 minute	2.5 minutes	
Staphylococcus aureus IFO 12732	−	−	−	−	−	−	−	−	+
MRSA (oxacillin: 1600 H/mL, clinical isolate)	−	−	−	−	−	−	−	−	+
MRSA (oxacillin: 12.5 µ g/mL, clinical isolate)	−	−	−	−	−	−	−	−	+
Staphylococcus epidermidis IFO 12993	−	−	−	−	−	−	−	−	+
Enterococcus faecalis IFO 12965	−	−	−	−	−	−	−	−	+
Staphylococcus hominis JCM 2419	−	−	−	−	−	−	−	−	+
Pseudomonas aeruginosa IFO 13275	−	−	−	−	−	−	−	−	+
Burkholderia cepacia IFO 14595	−	−	−	−	−	−	−	−	+
Serratia marcescens IFO 12648	−	−	−	−	−	−	−	−	+
Proteus vulgaris IFO 3988	−	−	−	−	−	−	−	−	+
Klebsiella pneumoniae IFO 3317	−	−	−	−	−	−	−	−	+
Salmonella typhi TD	−	−	−	−	−	−	−	−	+
Escherichia coli IFO 3806	−	−	−	−	−	−	−	−	+
Enterobacter cloacae IFO 13535	−	−	−	−	−	−	−	−	+
Bacillus subtilis IFO 3134 (spore type)	+	+	−	−	+	+	+	−	+

+: Surviving
-: Dead
31) Sakagami, Y. et al., Journal of Antibacterial and Antifungal Agents, 26(11), pp. 605-610, 1998. (In Japanese)

Table 6: Bactericidal activities against *Mycobacterium tuberculosis* and atypical acid-fast bacteria

	Acecide active solution						Control
	15 seconds	30 seconds	1 minute	2.5 minutes	5 minutes	10 minutes	
M. tuberculosis H37Rv	+	±	−	−	−	−	+
M. avium ATCC15769	+	−	−	−	−	−	+
M. intracellulare ATCC13950	+	−	−	−	−	−	+
M. kansasii ATCC25414	−	−	−	−	−	−	+
	2.0% glutaraldehyde solution						Control
	15 seconds	30 seconds	1 minute	2.5 minutes	5 minutes	10 minutes	
M. tuberculosis H37Rv	+	+	±	±	±	−	+
M. avium ATCC15769	+	+	±	±	±	±	+
M. intracellulare ATCC13950	+	+	+	−	−	−	+
M. kansasii ATCC25414	+	±	−	−	−	−	+

+: Surviving -: Dead ±: Surviving or dead

Table 7: Bactericidal activity against various fungi

	Acecide active solution			2.0% glutaraldehyde solution		
	Control	5 minutes	10 minutes	Control	5 minutes	10 minutes
Aspergillus niger IFO 9455 (ATCC16404)	+	−	−	+	−	−
Candida albicans IFO 1594 (ATCC10231)	+	−	−	+	−	−
Filobasidiella neoformans OPS 304	+	−	−	+	−	−
Trichophyton mentagrophytes IFO 32412	+	−	−	+	−	−

+: Surviving -: Dead

Table 8: Inactivation of various viruses

	Acecide active solution			2.0% glutaraldehyde solution		
	Control	2.5 minutes	5 minutes	Control	2.5 minutes	5 minutes
Adeno virus type 5	+	−	−	+	−	−
Herpes simplex virus type 1	+	−	−	+	−	−
Polio virus type 3	+	+	−	+	±	±

+: Surviving -: Dead ±: Surviving or dead

(6) Sporicidal activity curve

Sporicidal activity of Acecide active solution was examined by exposure within 5 minutes and the time taken for reducing the indicator bacteria (*B. subtilis* spore) by 12 logs (12D) (reduction from the initial microbial count of 10^6 to the sterility assurance level of 10^{-6}) according to the overkill method.
By extrapolating the plot obtained (Fig. 3), the time taken for eradication to 10^{-6} was 2.79 minutes with 0.3% Acecide active solution and 7.86 minutes with 0.2% Acecide active solution.
Thus, it was found that Acecide active solution met the requirement to achieve 12-log (12D) decrease by 10-minute exposure at a concentration below the minimum recommended concentration (MRC) of 0.2% PAA.

Method

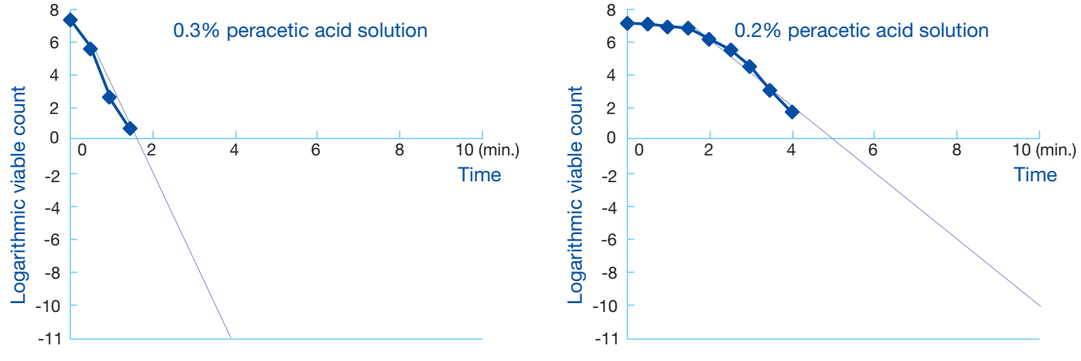
i) 1 mL of the test microbial solution (*B. subtilis* spore: 1.4-2.2 x 10^7 CFU/mL) was inoculated to 9 mL of the test solution (Acecide active solution containing 0.3% or 0.2% peracetic acid) kept at 20ºC.

ii) After a designated time of exposure, the test substance was inactivated with 0.5% sodium thiosulfate solution and 1 mL of the solution or 1 mL of the serial dilution (with sterilized purified water) was mixed with the medium and incubated.

iii) After incubation at 37ºC for 48 hours, live bacteria were counted (viable cell count).

iv) The logarithmic viable cell count was plotted against time and the exposure time to achieve 12-log reduction was determined by extrapolating the approximate line.

Figure 3: Relationship between the logarithmic viable count of *B. subtilis* spores after exposure to the Acecide active solution (containing various concentrations of Peracetic acid) and exposure time



7. Safety Information

1. Notes#

i) Acecide is a high-level disinfectant for reprocessing the Olympus flexible endoscopes with the OER-AW.
ii) Acecide is designed to be prepared by mixing Reagent One and Reagent Two in the OER-AW as directed in the instruction manual.
iii) Acecide can be reused until the active solutions concentration is equal to or greater than the minimum effective concentration. Before use, confirm that the concentration is above 0.2% by monitoring an Acecide Test Strip.

2. Safety Instructions*

i) Avoid contact with skin.
ii) Do not swallow. Store and handle carefully according to label instruction and MSDS.
iii) Ensure adequate ventilation during storage and handling.
iv) Wear protective glasses and gloves to avoid splash contact and vapor contact to eyes. If contact to eyes occurs, flush with water and seek medical attention.
v) Wear protective mask to avoid vapor inhalation. Seek medical attention if shortness of breath or prolonged coughing occurs.
vi) DO NOT open the bottles manually.

3. First aid*

i) EYE (CONTACT):
Hold affected eyes open and flush with water for at least 10-15 minutes. Seek medical attention without delay.
ii) SKIN (CONTACT):
Remove contaminated clothing. Wash skin with water thoroughly and seek medical attention.
iii) INHALATION (BREATHING)
Move to a well-ventilated area immediately without delay. Seek medical attention.
iv) INGESTION (SWALLOWING)
DO NOT induce vomiting. Give water only if patient is conscious. Seek medical attention immediately.

4. Storage*

i) Store in a cool dry place (0-25°C) and away from sunlight.
ii) The expiration date of Acecide is found on the immediate container.

5. Disposal*

i) Dispose according to local, state and national standards, guidelines, and regulations.

#: Please refer to the instruction manual provided with this product for complete inspection criteria, warnings, cautions and instructions for use.
*: Please refer to the MSDS for Acecide Reagent 1 and Acecide Reagent 2 for complete details on Safety Instructions, First Aid, Storage and Disposal.

OLYMPUS AUSTRALIA
31 Gilby Rd Mount Waverley VIC 3149 AUST
Ph. 1300 132 992
www.olympusaustralia.com.au

OLYMPUS NEW ZEALAND
15D Paul Matthews Rd Albany Auckland NZ
Ph. 0508 659 6787
www.olympus.co.nz