

TECHNICAL BRIEF

The Effect of Peracetic Acid on Steritest™ Sterility Testing Devices



Introduction

Closed, disposable Steritest sterility test devices reduce the risk of false positives during the sterility testing of pharmaceuticals. To further reduce the risk of false positives, some manufacturers use the devices inside an isolator. Typically, the filtration devices are placed in the isolator while the isolator is decontaminated using sterilization gases.

The purpose of this study was to compare the fertility of standard Steritest devices with HA membrane (TLHALV210) after exposure to a peracetic acid atmosphere in an isolator to non-exposed TLHALV210 Steritest units.

The study was conducted in two phases:

- In Phase 1, we compared the fertility of non-exposed Steritest TLHALV210 units to Steritest TLHALV210 units exposed to a peracetic acid atmosphere for 6 hours followed by 12 hours flushing.
- In Phase 2, we again compared exposed and non-exposed units but pre-rinsed each canister with 50 mL of sterile peptone solution to determine the effect of membrane pre-rinsing on the elimination of peracetic acid residues.

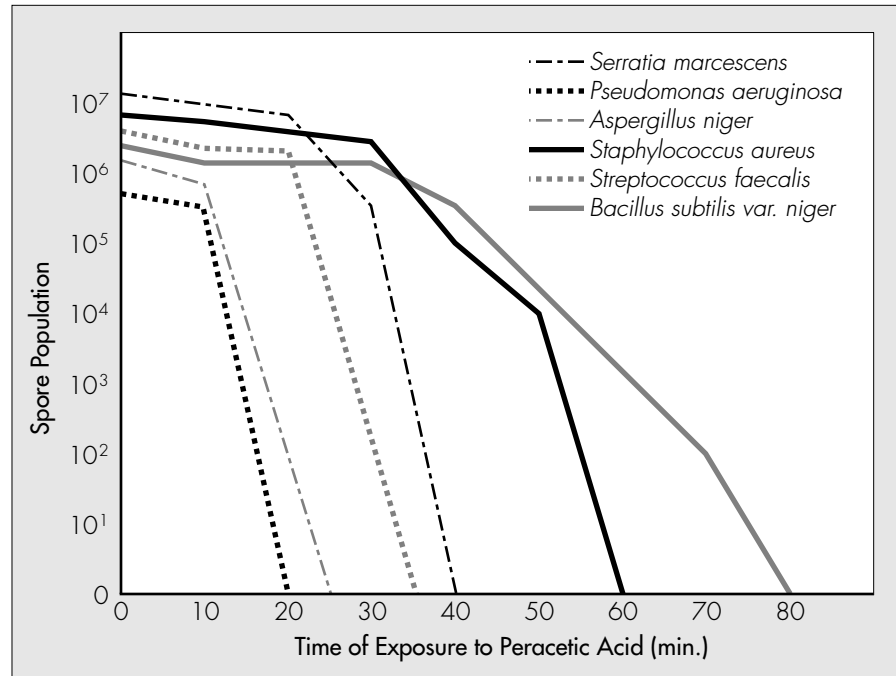


Figure 1. *Pseudomonas aeruginosa* shows little resistance to peracetic acid.

Pseudomonas aeruginosa was selected for the fertility tests because of the microorganism's high sensitivity to peracetic acid. (Figure 1). The fertility tests were conducted by:

- Inoculating 10 and 100 germs into 100 mL of broth and transferring the broth into Steritest canisters.
- Inoculating 10 and 100 germs into 10 mL of agar medium and transferring the medium into Steritest units with colony counts.

- Inoculating 10 or 100 germs into a solution, filtering the solution through the Steritest units, and then cutting the membranes from the canisters and placing them in Petri dishes with colony counts.*

* Because *Pseudomonas aeruginosa* is an aerobic germ, we assumed that its growth can be inhibited in agar medium. Therefore, we conducted this experiment in Phase 1 to determine whether we obtained the same counts with a membrane aerated in a Petri dish. The Phase 1 experiment showed no significant difference in colony counts between the two methods after 7 days incubation and it was decided to keep just the agar medium method for Phase 2.

Experimental Design

Phase 1

Growth promotion of Steritest units TLHALV210 versus Steritest TLHALV210 exposed to a peracetic atmosphere.

| | Sterility Control | Fertility at ~ 10 CFU | Fertility at ~ 100 CFU |
|---|---|--|--|
| ETO-Sterilized Steritest | 1 Unit 1 canister with 100 mL broth 1 canister with ~ 10 mL agar medium (colony count) | 10 Units 10 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) 5 canisters with membrane cut out and placed in a Petri dish (colony count) | 10 Units 10 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) 5 canisters with membrane cut out and placed in a Petri dish (colony count) |
| ETO-Sterilized Steritest + 6 Hours Isolator + 12 Hours Flushing | 1 Unit 1 canister with 100 mL broth 1 canister with ~ 10 mL agar medium (colony count) | 10 Units 10 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) 5 canisters with membrane cut out and placed in a Petri dish (colony count) | 10 Units 10 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) 5 canisters with membrane cut out and placed in a Petri dish (colony count) |

Phase 2

Growth promotion of Steritest devices versus Steritest units exposed to a peracetic atmosphere followed by a pre-rinsing step with 50 mL sterile peptone solution per canister.

Pre-Rinsing with 50 mL Sterile Peptone Solution Per Canister

| | Sterility Control | Fertility at ~ 10 Germs | Fertility at ~ 100 Germs |
|---|---|---|---|
| ETO-Sterilized Steritest | 1 Unit (with pre-rinsing step) 1 canister with 100 mL broth 1 canister with ~ 10 mL agar medium (colony count) | 5 Units 5 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) | 5 Units 5 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) |
| ETO-Sterilized Steritest + 6 Hours Isolator + 12 Hours Flushing | 1 Unit (with pre-rinsing step) 1 canister with 100 mL broth 1 canister with ~ 10 mL agar medium (colony count) | 5 Units 5 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) | 5 Units 5 canisters with 100 mL broth 5 canisters with ~10 mL agar medium (colony count) |

Materials and Methods

Materials

The following materials were used:

- La Calhène Isolator
- ETO-sterilized Steritest filtration devices in blister packages (Millipore, cat. no. TLHALV210 from one lot number)
- Biological indicators (spore strips)
- Indicator strips for determination of peracetic acid levels (Merckoquant, 5–50 mg, 1,10084,0001)

Methods

The isolator was loaded with Steritest units and 10 spore strips were placed inside the isolator taking into consideration the critical points of the isolator (e.g., in the corners of the isolator chamber and between the blister packages).

In addition, 12 sealed test tubes containing trypticase soy medium were placed in the isolator before the sterilization cycle (10 for the 10 spore strips, one for sterility control, one for fertility control).

After the sterilization cycle and flushing, spore strips were placed into 10 tubes (one spore strip per tube) and the tubes were incubated. The sterility control tube remained closed and the fertility control tube was incubated with an unsterilized spore strip from the same lot.

These steps were performed to ensure the efficacy of the isolator sterilization cycle.

Two additional sealed tubes were also included: one with a dry peracetic acid indicator strip and one with a sterile peptone solution used for wetting the peracetic acid indicator strip before reading.

Sterilization of the isolator

The isolator was connected to an automated sterilizer. The volume of peracetic acid required for sterilization was 50mL/hour plus 150 mL residual volume. The sterilization time was set at 6 hours with a timer. The peracetic acid flow rate (as vapor) was adjusted to 45 to 50 mL per minute. The pressure within the isolator was set at 6 to 7 mm water.

Flushing of the isolator

The 12 hour flushing phase was automatically activated after the sterilization phase. Flushing was ensured by the circulation of filtered air through the bubble. All manipulations were then performed by the operator using the glove box under constant filtered airflow (flow rate adjusted to 6 to 7 mm of water).

Confirming isolator sterility after flushing

The spore strips confirmed the capability of peracetic acid to kill *Bacillus subtilis* spores (10^6). The spore strips previously inserted in various locations of the isolator were put into 10 test tubes containing trypticase soy broth. The tubes were incubated for 14 days at 37 °C (10 test tubes with spore strips, one fertility control, and one sterility control).

Confirming acidity levels after flushing

After the isolator was flushed with air, the tubes containing the Merckoquant 5–50 mg peracetic acid indicator strips were opened, the pads were wetted, and readings were performed after exposure to the isolator atmosphere for 5 seconds. The acidity within the isolator was to remain at or below 5 ppm.

Results and Discussion

Peracetic Acid Levels Inside the Isolator

The Merckoquant 5–50 mg peracetic acid indicator strips indicated that the peracetic acid level remained <5 ppm after each sterilization cycle.

Isolator Load Sterility

Isolator sterilization was effective because the 10 spore strips that were placed in different areas within the isolator showed no growth after 14 days incubation in the two sterilization cycles.

**Experiment 1:
Growth Promotion of Steritest
Units TLHALV210 Versus Steritest
TLHALV210 Exposed to a
Peracetic Atmosphere**

At about 10 CFU, the Steritest units exhibited total growth inhibition in 30% of the cases in liquid medium after 7 days and total growth inhibition in 80% of the cases on solid

medium (10 mL agar medium in the canister or membrane cut out and placed in a Petri dish).

At about 150 CFU, the Steritest units exhibited slightly delayed growth in liquid medium and a partial growth inhibition on solid medium (10 mL agar medium in the canister or membrane cut out and placed in a Petri dish). See Tables 1 and 2.

Table 1

| | | Fertility at 10 Germs | | | | | | |
|---|--------------------|-----------------------|-------------------|---|-----------------|--|-------|---|
| | | In 100 mL Broth | | In 10 mL Agar Medium (Colony Count as CFU) | | With Membrane Cut Out and Placed in a Petri Dish (Colony Count as CFU) | | |
| | | Day 1 | Day 7 | Day 1 | Day 7 | Day 1 | Day 7 | |
| EO-Sterilized Canisters (20 canisters) | 1 | + | + | 0 | 26 | / | / | |
| | 2 | + | + | 0 | 19 | / | / | |
| | 3 | + | + | 0 | 15 | / | / | |
| | 4 | + | + | 0 | 19 | / | / | |
| | 5 | + | + | 0 | 10 | / | / | |
| | 6 | + | + | / | / | 15 | 13 | |
| | 7 | + | + | / | / | 14 | 14 | |
| | 8 | + | + | / | / | 20 | 19 | |
| | 9 | + | + | / | / | 15 | 15 | |
| | 10 | + | + | / | / | 13 | 13 | |
| Negative Control | | (-) | (-) after 14 days | 0 | 0 after 14 days | / | / | |
| EO-Sterilized Canisters (20 canisters) | 1 | - | - | 0 | 0 | / | / | |
| | 2 | - | - | 0 | 0 | / | / | |
| | 3 | + | + | 2 | 16 | / | / | |
| | 4 | Weak | + | 0 | 0 | / | / | |
| | 6 Hrs. Exposure PA | 5 | Weak | + | 0 | 0 | / | / |
| | | 6 | - | + | / | / | 0 | 0 |
| | 12 Hrs. Flushing | 7 | Weak | + | / | / | 0 | 1 |
| | | 8 | - | + | / | / | 0 | 0 |
| | | 9 | Weak | + | / | / | 0 | 0 |
| | | 10 | - | - | / | / | 0 | 0 |

Table 2

| | | Fertility at 100 Germs | | | | | | |
|---|--------------------|------------------------|-------|---|-------|--|-------|----|
| | | In 100 mL Broth | | In 10 mL Agar Medium (Colony Count as CFU) | | With Membrane Cut Out and Placed in a Petri Dish (Colony Count as CFU) | | |
| | | Day 1 | Day 7 | Day 1 | Day 7 | Day 1 | Day 7 | |
| EO-Sterilized Canisters (20 canisters) | 1 | + | + | 5 | 176 | / | / | |
| | 2 | + | + | 1 | 146 | / | / | |
| | 3 | + | + | 3 | 184 | / | / | |
| | 4 | + | + | 5 | 139 | / | / | |
| | 5 | + | + | 0 | 163 | / | / | |
| | 6 | + | + | / | / | 188 | 166 | |
| | 7 | + | + | / | / | 162 | 157 | |
| | 8 | + | + | / | / | 178 | 162 | |
| | 9 | + | + | / | / | 184 | 166 | |
| | 10 | + | + | / | / | 189 | 181 | |
| EO-Sterilized Canisters (20 canisters) | 1 | + | + | 0 | 0 | / | / | |
| | 2 | Weak | + | 0 | 0 | / | / | |
| | 3 | + | + | 0 | 26 | / | / | |
| | 4 | + | + | 0 | 9 | / | / | |
| | 6 Hrs. Exposure PA | 5 | + | + | 0 | 0 | / | / |
| | | 6 | Weak | + | / | / | 0 | 0 |
| | 12 Hrs. Flushing | 7 | + | + | / | / | 1 | 1 |
| | | 8 | Weak | + | / | / | 0 | 0 |
| | | 9 | + | + | / | / | 0 | 10 |
| | | 10 | + | + | / | / | 0 | 1 |

**Experiment 2:
Growth Promotion of Steritest Units
Versus Steritest Units Exposed to a
Peracetic Atmosphere Followed by a
Pre-rinsing Step with 50 mL Sterile
Peptone Solution Per Canister**

The Steritest units exhibited good growth after 1 day and 7 days in liquid medium and on solid medium (10 ml agar medium in the canister) at both about 5 and 50 CFU (Tables 3 and 4).

Conclusion

The fertility tests done with Steritest units using *Pseudomonas aeruginosa*, which is known to be highly sensitive to peracetic acid, showed no evidence of inhibitory residuals after rinsing the membrane with 50 mL of sterile peptone solution immediately after peracetic acid sterilization within the isolator.

These conclusions are only valid for the conditions evaluated in this study.

Each isolator must be validated independently because each isolator's configuration, contents, and sterilization cycle protocols vary.

If growth inhibition is detected during validation of a sterility testing procedure within an isolator sterilized with peracetic acid, Millipore recommends that customers incorporate and optimize a pre-rinsing step of the Steritest canisters before contact with pharmaceutical product.

Table 3

| | | Fertility at 10 Germs Pre-Wetting (50 mL/Canister) | | | |
|---|--------------------|--|-------------------|---|-----------------|
| | | In 100 mL Broth | | In 10 mL Agar Medium (Colony Count as CFU) | |
| | | Day 1 | Day 7 | Day 1 | Day 7 |
| EO-Sterilized Canisters (10 canisters) | 1 | + | + | + | 5 |
| | 2 | + | + | + | 7 |
| | 3 | + | + | + | 7 |
| | 4 | + | + | + | 9 |
| | 5 | + | + | + | 8 |
| Negative Control (with pre-rinsing) | | (-) | (-) after 14 days | 0 | 0 after 14 days |
| EO-Sterilized Canisters (10 canisters) | 1 | + | + | + | 6 |
| | 2 | + | + | + | 4 |
| | 3 | + | + | + | 7 |
| | 6 Hrs. Exposure PA | 4 | + | + | 6 |
| | 12 Hrs. Flushing | 5 | + | - | 7 |

Table 4

| | | Fertility at 100 Germs Pre-Wetting (50 mL/Canister) | | | |
|---|-------------------|---|-------|---|-------|
| | | In 100 mL Broth | | In 10 mL Agar Medium (Colony Count as CFU) | |
| | | Day 1 | Day 7 | Day 1 | Day 7 |
| EO-Sterilized Canisters (10 canisters) | 1 | + | + | + | 79 |
| | 2 | + | + | + | 70 |
| | 3 | + | + | + | 54 |
| | 4 | + | + | + | 53 |
| | 5 | + | + | + | 63 |
| EO-Sterilized Canisters (10 canisters) | 1 | + | + | + | 53 |
| | 2 | + | + | + | 64 |
| | 3 | + | + | + | 70 |
| | 6 Hrs Exposure PA | 4 | + | + | 66 |
| | 12 Hrs. Flushing | 5 | + | - | 58 |

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:

In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**

In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**

Internet: www.millipore.com

E-mail: tech_service@millipore.com

Millipore Worldwide

AUSTRALIA Millipore Australia Pty. Ltd./9A Byfield Street/North Ryde/NSW 2113/Tel. 1 800 222 111 (toll free) or (02) 9888 8999/Fax (02) 9878 0788

AUSTRIA Millipore Ges.m.b.H./Hietzinger Hauptstrasse 145/A-1130 Wien/Tel. (01) 877-8926/Fax (01) 877-1654

BALTIC COUNTRIES Millipore Oy/Ruukinkuja 4/FIN-02320 Espoo, Finland/Tel. +358 9 804 5110/Fax +358 9 256 5660

BELGIUM AND LUXEMBOURG Millipore S.A.-N.V./Rue de la Fusée 60/Raketstraat 60/B-1130 Brussels/Tel. +32 2 726 88 40/Fax +32 2 726 98 84

BRAZIL Millipore Indústria e Comércio Ltda./Rua Prof. Campos de Oliveira, 430/CEP 04675-100/Saõ Paulo-SP/Tel. (011) 5548-7011/Fax (011) 5548-7923

CANADA Analytical Division: Millipore (Canada) Ltd./36 Antares Drive, Suite 280/Nepean, Ontario K2E 7W5/Tel. 1-800-645-5476/Fax 1-800-645-5439

BioProcess Division: Millipore (Canada) Ltd./109 Woodbine Downs Blvd, Unit 5/Etobicoke, Ontario M9W 6Y1/Tel. 1-800-645-5476/Fax 1-800-645-5439

Laboratory Water Division: Millipore (Canada) Ltd./19 Thorne St. Suite 302/Cambridge, Ontario/N1R 1S3/Tel. 1-800-645-5476/Fax 1-800-645-5439

CHINA, PEOPLE'S REPUBLIC OF

Beijing: Millipore China Ltd./Suite 1209 China Resources Building/8 Jianguomenbei Avenue/Beijing 100005/Tel. (8610) 8519 1250, (8610) 6518 1058/
Fax (8610) 8519 1255

Guangzhou: Millipore China Ltd./Suite 1303 Office Tower, Citic Plaza/233 Tian He Bei Road/Guangzhou 510620/Tel. (8620) 8755 4049/
Fax.(8620) 8752 0172

Hong Kong: Millipore Asia/China Ltd./Suite 3201 Central Plaza/18 Harbour Road, Wanchai, Hong Kong/Tel. (852) 2803 9111/Fax (852) 2513 0313

Shanghai: Millipore China Ltd./Suite 901 Hong Kong Plaza (S)/283 Huai Hai Road (M)/Shanghai 200021/Tel. (8621) 5306 9100/Fax (8621) 5306 0838

CZECH REPUBLIC Millipore spol. s.r.o./Ricanova 21/16900 Praha 6/Tel. 02-2051 3841/ 02-2051 3842/Fax 02-2051 4298

DENMARK Millipore A/S/Odinsvej 9-19, st./2600 Glostrup/Tel. 70 10 00 23/Fax 70 10 13 14

EASTERN EUROPE, C.I.S., AFRICA, MIDDLE EAST AND GULF

Analytical Division: Millipore S.A./BP 116/67124 Molsheim Cedex, France/Tel. +33 3.88.38.9536/Fax +33 3.88.38.9539

BioProcess Division: Millipore Ges.m.b.H./Hietzinger Hauptstrasse 145/A-1130 Wien, Austria/Tel. +43 1 877-8926/Fax +43 1 877-1654

Laboratory Water Division: Millipore S.A./BP 307/78054 Saint-Quentin/Yvelines Cedex, France/Tel. +33 1.30.12.70.00/Fax +33 1.30.12.71.80

FINLAND Millipore Oy/Ruukinkuja 4/02320 Espoo/Tel. (09) 804 5110/Fax (09) 256 5660

FRANCE Millipore S.A./BP 307/78054 Saint-Quentin/Yvelines CEDEX/Tel. (01) 30.12.70.00/Fax (01) 30.12.71.80

GERMANY Millipore GmbH/Hauptstraße 87/65760 Eschborn/Tel. (06196) 494-0/Fax (06196) 43901

HUNGARY Millipore Kft./Andor u. 47-49/1119 Budapest/Tel. 1-205 9784/Fax 1-205 9792

INDIA Millipore (India) Pvt. Ltd./50A 2nd Phase Ring Road/Peenya/560 058 Bangalore/Tel. (91) 80-839 46 57/Fax (91) 80-839 63 45

ITALY Milano: Millipore S.p.A./Via XI Febbraio, 99/20090 Vimodrone/Tel. (02) 25.07.81/Fax (02) 26.50.324

Roma: Millipore S.p.A./Via D. Sansotta 100/00144 Roma/Tel. (06) 52.03.600/Fax (06) 52.95.735

JAPAN Nihon Millipore Ltd./Mita Kokusai Bldg./4-28, Mita 1-Chome/Minato-ku/Tokyo 108/Tel. (03) 5442-9711/Fax (03) 5442-9736 (Analytical Division)/
Fax (03) 5442-9737 (BioProcess Division)/Fax (03) 5442-9734 (Laboratory Water Division)

KOREA Millipore Korea Co. Ltd./Suite 711, Korea City Air Terminal Building/159-6, Samsung-Dong, Kangnam-Ku/Seoul 135-728/Tel. (822) 551-0990/
Fax (822) 551-0288

MALAYSIA Millipore Asia Ltd./Suite 3.03 Wisma KT/No. 14 Jalan 19/1/46300 Petaling Jaya/Selangor/Tel. 603-7571322/Fax 603-7571711

MEXICO Millipore S.A. de C.V./Ings. Militares 85-PB/Col. Argentina Poniente/11230 México, D.F./Tel. (525) 576-9688/Fax (525) 576-8706

THE NETHERLANDS Millipore B.V./Penningweg 33/Postbus 166/4870 AD Etten-Leur (N.B.)/Tel. 076-5022000/Fax 076-5022436

NORWAY Millipore AS/Postboks 214 - Manglerud/0612 Oslo/Tel. 22 67 82 53/Fax 22 66 04 60

POLAND Millipore Sp.z.o.o./ul. Jasniodvorska 7/01745 Warszawa/Tel. 22-669 12 25/ 22-663 70 31/Fax 22-663 70 33

PUERTO RICO Millipore Cidra Inc./P.O. Box 11977/Cidra, P.R. 00739-1977/Tel. (787) 273-8495/Fax (787) 747-6553

SINGAPORE Millipore Singapore Pte Ltd./31 Kaki Bukit Road 3/#06-08/11 Techlink/Singapore 417818/Tel. (6 5) 842 1822/Fax (65) 842 4988

SPAIN AND PORTUGAL

Madrid: Millipore Iberica, S.A./Avda. Ilano Castellano,13-3º/28034 Madrid/Tel. 917 283 960/Fax 917 292 909

Barcelona: Millipore Ibérica, S.A./Balmes, 89-91, 8º/08008 Barcelona/Tel. 934 525 530/Fax 934 516 048

SWEDEN Millipore AB/Box 7090/174 07 Sundbyberg/Tel. 08-628 6960/Fax 08-628 6457

SWITZERLAND

Volketswil: Millipore AG/Chriesbaumstrasse 6/8604 Volketswil/Tel. (01) 908-30-60/Fax (01) 908-30-80

Lausanne: Millipore S.A./43, Chemin de Maillefer/1052 Le Mont-sur-Lausanne/Tel. (021) 641 2550/Fax (021) 641 2551

TAIWAN Taipei: Millipore Asia Ltd./Unit #707, 7th fl/136 Sec.3 Jen-Ai Road/Taipei 10628 /Tel. (886-2) 700-1742/Fax (886-2) 755-3267

Hsin Chu City: Millipore Asia Ltd./2F., No. 8, Lane 99/Pu-Ding Road/Hsin Chu City/Tel. (886-3) 571-0178/Fax (886-3) 572-9520

U.K. AND IRELAND Millipore (U.K.) Ltd./The Boulevard/Blackmoor Lane/Watford, Hertfordshire WD1 8YW/Tel. +44 1923 816375/Fax +44 1923 818297

U.S.A. Millipore Corporation/80 Ashby Road/Bedford, MA 01730-2271/Tel. (781) 533-6000/Fax (781) 533-3110

In All Other Countries

MILLIPORE INTERTECH P.O. Box 255 /Bedford, MA 01730 U.S.A./Tel. +1 (781) 533-8622/Fax +1 (781) 533-8630

Millipore, Integritest, and Steritest are trademarks of Millipore Corporation or an affiliated company.

ATCC is a trademark of American Type Culture Collection.

Lit. No. TB1012EN00 Printed in U.S.A. 1/01 00-307

Copyright 2000 Millipore Corporation or an affiliated company, Bedford, MA. All rights reserved.

MILLIPORE