ATTACHMENT 3

CERTIFICATE OF PLANT DESIGN REGISTRATION

Occupational Health & Safety Act 2000
Occupational Health & Safety Regulation 2001

ABN: 77 682 742 966
Phone: (02) 4321 5498
Fax: (02) 4325 5094

Registration No: PV 6-153790/11 ABN: 70077391541 Issue Date: 22/11/2011

Controller: MEDIVAC TECHNOLOGY PTY LTD
Postal Address: PO BOX 656
BAULKHAM HILLS
NSW 1755

Plant Type: Pressure Vessel Original

Design Description:

Quality System	No
Hazard Level	C
Contents	Harmful
Chamber 1 Volume (l)	644
Chamber 1 Design Pressure (kPa)	-100 TO 350
Chamber 1 Temperature (°C)	148
Chamber 1 Fluid Type	Gas
Chamber 2 Volume (l)	21
Chamber 2 Design Pressure (kPa)	400
Chamber 2 Temperature (°C)	152
Chamber 2 Fluid Type	Gas
Drawing Number & Revisions	001.0000.00000 REV C
Pressure Vessel	Other
Other Type	STEAM JACKETED PRESSURE VESSEL

CONDITIONS:

1. This registration applies only to the design described above which has been notified to WorkCover NSW in accordance with the OHS Regulation 2001.
2. The plant owner will require a copy of this certificate. A copy of the certificate must therefore be supplied to the manufacturer so that it can, in turn, be provided to the supplier and owner with the item of plant or equipment.
3. WorkCover NSW reserves the right to audit the registered design at any time to assess compliance with its Acts and Regulations. If an audit is undertaken, detailed information may be requested relating to the design of the plant. Design systems of work and documentation may also be audited. If an audit identifies non-compliance, all plant built to that design may require modifications, and in some cases, may be prohibited from use.
4. This Registration is automatically invalidated if the design is altered to an extent that requires new measures to control risks. A person must not use, or cause or allow plant manufactured to the original design to be used at a workplace unless notification of the alteration, or the prescribed form, has been confirmed by WorkCover NSW.
5. The Registration Number should be quoted in all correspondence to WorkCover regarding this item. Any queries should be addressed to WorkCover's Licensing Unit.

Fee Paid: $ 130.00 Receipt No: 09-2317

CERTIFICATE OF PLANT DESIGN REGISTRATION

Occupational Health & Safety Act 2000
Occupational Health & Safety Regulation 2001

ABN: 77 682 742 966
Phone: (02) 4321 5498
Fax: (02) 4325 5094

Registration No: **BOIL 6-153791/11** ABN: 70077391541 Issue Date: **17/11/2011**

Controller: MEDIVAC TECHNOLOGY PTY LTD
Postal Address: PO BOX 656
BAULKHAM HILLS
NSW 1755

Plant Type: Boiler Alteration

Design Description:

Hazard Level	B
Design Pressure (kPa)	1400
Volume (l)	78
Temperature (Co)	198
Drawing Number and Revisions	102.0601.00000 REV A
Boiler Type	Electric
The Boiler Produces?	Steam

CONDITIONS:

1. This registration applies only to the design described above which has been notified to WorkCover NSW in accordance with the OHS Regulation 2001.
2. The plant owner will require a copy of this certificate. A copy of the certificate must therefore be supplied to the manufacturer so that it can, in turn, be provided to the supplier and owner with the item of plant or equipment.
3. WorkCover NSW reserves the right to audit the registered design at any time to assess compliance with its Acts and Regulations. If an audit is undertaken, detailed information may be requested relating to the design of the plant. Design systems of work and documentation may also be audited. If an audit identifies non-compliance, all plant built to that design may require modifications, and in some cases, may be prohibited from use.
4. This Registration is automatically invalidated if the design is altered to an extent that requires new measures to control risks. A person must not use, or cause or allow plant manufactured to the original design to be used at e workplace unless notification of the alteration, or the prescribed form, has been confirmed by WorkCover NSW.
5. The Registration Number should be quoted in all correspondence to WorkCover regarding this item. Any queries should be addressed to WorkCover's Licensing Unit.

Fee Paid: $ 130.00 **Receipt No: 09-2317**

NSW HEALTH

ENVIRONMENTAL HEALTH BRANCH

Mr Paul McPherson
Executive Chairman
Medivac Technology Pty Ltd
Unit 8, Lot 1B Kleins Road
NORTHMEAD NSW 2152

Ref: 02/3864

Dear Mr McPherson

NSW HEALTH APPROVAL OF MEDIVAC TECHNOLOGY CLINICAL WASTE TREATMENT DEVICE

I write in response to your email request for documentation of the approval of the 'Medivac Metamizer ML' with your current address details listed above.

This is to confirm that NSW Health issued an approval of the MediVac Technology Clinical Waste Treatment Device on 8 August 2002.

This letter of approval states that the *"MediVac Technology clinical waste treatment device which utilizes steam sterilization and a grinding process to reduce the bacterial and viral loads and spore loads of treated waste to levels of log 6 and log 4 respectively has been approved by the A/Director-General of NSW Health Department. The approval is for the treatment of certain types of clinical waste subject to conditions set out in schedule 1".*

In addition a letter was issued by NSW Health on 29 October 2004 advising that the Medivac MetaMizer ML is considered to be a *"new model to extend to the range of clinical waste treatment devices manufactured by MediVac because it uses the same technology and therefore comes under the existing approval granted by the Director-General on 15 July 2002".*

I hope this information is satisfactory.

Should you require any additional information in regard to the MediVac clinical waste treatment device approvals, please contact Ms Anne Ford A/Manager General Environmental Health on Tel (02) 9816 0225 or Email anne.ford@doh.health.nsw.gov.au.

Yours sincerely

Professor Wayne Smith
Director Environmental Health Branch
27 November 2008

NSW Department of Health
ABN: 92 697 899 630

PO Box 798 Gladesville NSW 1675
Building 11, Gladesville Hospital
Victoria Road, Gladesville NSW 2111
Telephone (02) 9816 0234 Fax (02) 9816 0240
Website www.health.nsw.gov.au

Tab 33

NSW HEALTH
DEPARTMENT

ENVIRONMENTAL HEALTH BRANCH

OUR FILE: 02/3864

Mark Butler
Managing Director
Medivac Technology Pty Ltd
PO Box 478
CASTLE HILL NSW 2154

Dear Mr Butler

NSW HEALTH APPROVAL OF MEDIVAC TECHNOLOGY CLINICAL WASTE TREATMENT DEVICE

Reference is made to your application for approval of the Medivac Technology clinical waste treatment device.

The Medivac Technology clinical waste treatment device which utilizes steam sterilization and a grinding process to reduce the bacterial and viral loads, and spore loads of treated waste to levels of log 6 and log 4 respectively has been approved by the A/Director-General of NSW Health Department. The approval is for the treatment of certain types of clinical waste subject to the conditions set out in Schedule 1.

The treated waste will need to be reclassified in accordance with EPA requirements before it can be disposed to landfill.

New systems or technologies that vary to this approval will need to be re-submitted for consideration.

If you would like to discus the approval please contact Nicole Badger on 98160225 or email nbadg@doh.health.nsw.gov.au.

Yours faithfully,

Neil Shaw
Manager, General Environmental Health
8/8/02.

PO Box 798. Gladesville NSW 1675
Administration Building. Gladesville Hospital
Victoria Road. Gladesville NSW 2111
Telephone (02) 9816 0373 Fax (02) 9816 0377

Commercial in Confidence

FINAL STUDY REPORT

MICROBIOLOGICAL REVALIDATION OF "METAMIZER ML SERIES II" WASTE STERILISER

Sponsor:
Mr Paul Woodford
Medivac Technology Limited
Unit 8 / Lot 1B Kleins Road
Northmead NSW 2152
Australia

Conducted by
AMS Laboratories Pty. Ltd.
8 Rachael Close,
Silverwater NSW 2128
Australia

Authors
Dr Paul Priscott, MSc, PhD
Ngoc Anh-Thu Phan Bsc.

Report reference no: 0905497
26th May 2009

EXECUTIVE SUMMARY

The study provides evidence that the MetaMizer ML Series II evaluation machine met the performance criteria for autoclave sterilization using the destruction of biological indicators (BI) as the measure of success. The revalidation was required due to modifications on the build material.

STUDY DIRECTORS STATEMENT

The study was conducted according to the procedures indicated by the sponsor.

To the best of my knowledge and belief, the study was conducted to Client specifications, and there were no circumstances that may have changed the quality and integrity of the study without prior knowledge of client.

Signed ..... Date 29/5/09

Dr Paul Priscott, MSc, PhD, MASM
Study Director

QUALITY ASSURANCE STATEMENT

The study was conducted in accordance with the OECD Principles on Good Laboratory Practice (as revised in 1997) and ISO /IEC 17025.

I certify that the data contained in this report is a true and accurate record of the experimental results.

Signed ..... Date 29/5/09

Ngoc Anh-Thu Phan Bsc, MASM
Quality Assurance Unit

ANALYSTS STATEMENT

The work reported herein is a true and accurate account of the results obtained in carrying out the stated procedures.

Signed---------- Date 29.05.09

Minal Patel
Microbiologist

LABORATORY CREDENTIALS

AMS Laboratories is licensed by the Australian Therapeutic Goods Administration (Licence Number: MI-15112007-LI002191-11) for microbiological analysis and testing and similarly by the Australian Pesticides and Veterinary Medicines Authority (Licence Number: 6139). The laboratory is also certified to ISO 17025:2005 (through NATA registration no. 15773) for its laboratory and company quality control systems.

CONFIDENTIALITY

The data and contents of this report are held in confidence by AMS Laboratories Pty Limited. They will only be made available to the Sponsor and authorized government inspectors if requested. No further disclosures will be made without seeking and receiving the prior permission of the Sponsor in writing.

STORAGE OF RECORDS

All materials, methods, variations to this protocol and results are recorded on laboratory worksheets. These records have been attached to a copy of this report and will remain archived at AMS Laboratories for a minimum of 7 years.

STUDY REPORT

STUDY TITLE: Microbiological Revalidation of MetaMizer ML Series II Waste Steriliser.

SPONSOR: Medivac Technology Limited. Unit 8, lot 1B Kleins Road Northmead 2152

TEST FACILITY: AMS Laboratories Pty Ltd 8 Rachael Close Silverwater 2128

INTRODUCTION

A previous study (report 0405553) demonstrated the efficacy of the MetaMizer ML series II to sterilize waste using both BI strips and BI suspensions. Due to recent modifications to the machine, a study was required by Medivac Technology Ltd to revalidate the MetaMizer ML Series II using the spore organism *Geobacillus stearothermophilus* ATCC 7953 in suspension form, deemed to be the most stable for transport from site to the laboratory.

The MetaMizer ML Series II evaluation machine was operated by Medivac Technology staff on site. All BI suspensions were loaded and retrieved by AMS Laboratories staff.

OBJECTIVES

To ensure material modifications made to MetaMizer ML series II has not affected the process efficacy of the machine through the use of BI's during the sterilization process.

EXPERIMENTAL START DATE: 6th May 2009
STUDY COMPLETION DATE: 26th May 2009

TEST METHOD: On-Site Validation Protocol

TEST STRAINS:

Geobacillus stearothermophilus ATTC 7953

STUDY MATERIALS

MEDIA

Tryptone Soy Agar (TSA)

REAGENTS

Tryptone Soy Broth (TSB)

EQUIPMENT

Petri dishes 90mm
55° C Incubator, 30° C Incubator
Eppendorf pipettes and sterile disposable tips
Sterile disposable pipettes
Sterile MacCartney bottles
Sterile mixing sticks
Balance
Forceps

TEST METHOD

BIOLOGICAL INDICATORS

The evaluation organism in all BI's was *Geobacillus stearothermophilus* (previously known as *Bacillus stearothermophilus*). *G. stearothermophilus* strain ATCC 7953, was grown in-house to high numbers, heat treated to inactivate any vegetative (less resistant) forms of the organism, centrifuged and titrated to enumerate the challenge suspension. This spore suspension was inoculated onto green cotton strips, with one added to every MetaMizer ML Series II load in order to retrieve the inoculated material more precisely.

MATERIALS AND METHODS

On Site

100ml of the BI suspension was inoculated onto a distinct green cotton sheet and placed in a biohazard bag with various clinical wastes.

The MetaMizer ML Series II was initiated with a manual crunch cycle, no heat was applied to the system. This was the base line control cycle run. Waste samples were collected in a biohazard bag lined bin. The entire contents from the cycle was retrieved, tied and placed into an 'esky' for transportation back to the laboratory.

Three consecutive cycles were then run with heat applied for the sterilization process. As with the control run, 100ml of BI suspension was inoculated onto a distinct green cotton sheet. The clinical waste sample was retrieved as above.

Laboratory Procedures

For the control run, analysts immediately separated and weighed 10g of the green inoculated cotton material from the clinical waste bags. The sample was then serially diluted and plated in duplicate to assay the numbers of recovered spores.

The heat cycle samples were also processed with 10g of green inoculated cotton material being placed into 100ml of TSB media.

Unprocessed BI's suspensions were serially diluted and plated in duplicate to determine the titre used to inoculate each cycle (Table 1).

All plates and broths were incubated at 55°C for 72 hours. Plates were counted and averages determined and broths observed for presence or absence of turbidity (Table 2).

Waste Water Testing

After the final cycle, waste water from the run was collected and tested for Total Aerobic Plate count and presence of *G. stearothermophilus*. 100ml of water was filtered and the membrane placed onto TSA agar and incubated for 72 hours at 30°C for total aerobic count results, whilst 100ml was filtered and the membrane placed into 100ml of TSB and incubated at 55°C for 72 hours for the presence of *G. stearothermophilus* (Table 3).

RESULTS

BI Population Confirmation

Table 1. BI Vial Assay Results

***G. stearothermophilus* 100ml vial**	***G. stearothermophilus* Count cfu/ml**
Manual control	1.5×10^7
Heat cycle 1	1.3×10^7
Heat cycle 2	1.5×10^7
Heat cycle 3	1.4×10^7

Table 2. BI Test Results post MetaMizer ML series II cycles

Cycle	***G. stearothermophilus* Count cfu/g**
Manual	3.6×10^5
Heat Cycle 1	Not detected
Heat Cycle 2	Not detected
Heat Cycle 3	Not detected

Waste Water Evaluation

Table 3. Waste Water Growth Results

Test	Results cfu/100mls
Total Aerobic Plate Count	$> 8.0 \times 10^3$
G. stearothermophilus detection	Not detected

The results indicate the initial inocula were within the target range with counts being $1.3 \times 10^7 - 1.5 \times 10^7$. The manual cycle found the BI suspension retention number to be slightly lower at 3.6×10^5.

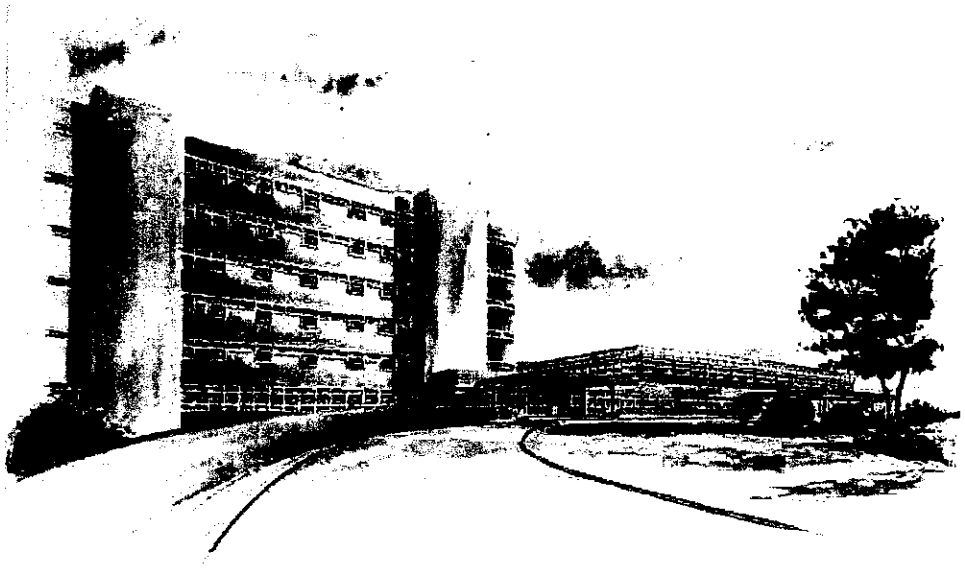
No BI was recovered from any of the three heat cycles, thus demonstrating a greater than 5 log reduction in BI presence.

Waste water sample results showed a high bacterial presence, however, no *G. stearothermophilus* was detected.

DISCUSSION AND CONCLUSION

The MetaMizer ML Series II was revalidated due to modifications made to the machine. One specially prepared BI was selected for processing through the cycle. *Geobacillus stearothermophilus* ATCC 7953 was selected as it is non-pathogenic to humans and animals yet is the most resistant to physical sterilization and is the internationally recognized standard organism for autoclave validations.

The results demonstrated that the MetaMizer ML Series II effectively sterilized the BI inoculated materials processed during the standard cycles.



REPORT ON THE MICROBIOLOGICAL EFFICACY TESTING OF THE CANNON HYGIENE MEDIVAC METAMIZER ML CLINICAL WASTE TREATMENT SYSTEM

November 2004

M G Holliday FIBMS, MSc, PhD, CSci, MBA
Microbiology Department
Freeman Hospital
Newcastle upon Tyne
UK

Prepared for:
Cannon Hygiene
Northgate House, Northgate, White Lund
Morecambe Lancashire LA3 3BJ

MG Holliday

Report Prepared by .....

CH/MET/MGH/1104/ver2

A) EXECUTIVE SUMMARY

A.1.1. The Cannon Hygiene MediVac MetaMizer ML clinical waste treatment plant at Northgate White Lund, Morecambe has achieved STAATT level IV inactivation when tested under normal operating conditions. The manufacturers' stated normal operating parameters for the programmable logic controller (PLC) are 139° to 142°C for a holding time of 4 minutes.

A1.2 Microbial efficacy tests were carried out using two different protocols, one using spore strips and one using spore suspension challenges. Both methods gave comparable results.

A.1.3. STAATT level IV is equivalent to 6 $\log_{10}$ inactivation of *Bacillus atrophaeus (subtilis)* spores and is 100 times the level of inactivation required in the UK for clinical waste treatment processes (STAATT level III which is equivalent to 4 $\log_{10}$ inactivation of *Bacillus atrophaeus (subtilis)* spores).

A.1.4. Environmental air testing was carried out prior to any clinical waste treatment to establish baseline levels of microbial contamination. These levels can then act as a benchmark for comparison with environmental monitoring during routine plant operation.

A.1.5. Air samples taken from designated points in and around the plant showed that in all cases the microbial levels found were below the levels felt to require further investigation or remedial action. No pathogenic microorganisms were found in any sample.

A.1.6. It is my opinion that the plant can safely, effectively and reproducibly treat clinical waste to make it safe for final disposal.

I) DISCUSSION

I.1. MICROBIOLOGICAL EFFICACY TESTING

I.1.1. Spore suspension challenge testing of the MetaMizer system over 5 separate cycles proved that the system is capable of achieving >6 log kill of *B atrophaeus* spores.

I.1.2. Spore suspension challenge testing is recommended for systems that have integral shredding systems and where the integrity of a spore container cannot be guaranteed [1,2,3].

I.1.3. The MetaMizer system is a new development however and, even though an internal shredder is an integral part of the process within the treatment chamber, there is sufficient headroom above the shredder blades to suspend a spore strip container and maintain its integrity.

I.1.4. Because the MetaMizer recirculates the shredded waste within a sealed and pressurised system, it can be argued that steady state conditions exist within the chamber, and it is irrelevant whether spores are circulating with the waste or suspended in the chamber, as identical treatment conditions will be encountered. The shredding does not kill spores, but grinds the waste into a form that is more readily penetrated, accelerating treatment by heat.

I.1.5. All 60 spore strip challenge tests analysed after treatment in the MetaMizer, produced results equivalent to >6 log kill.

I.1.6. Thus, it was demonstrated that the recommended spore suspension challenge tests and the spore strip challenge tests produce the same results, with both confirming that the system can achieve > 6 log spore kill.

I.1.7. These results also prove the validity of the spore strip test in assessing the efficacy of the MetaMizer in killing Micro-organisms.

I.1.8. The spore suspension challenge test is technically difficult, time consuming and expensive to perform. It would be outside the scope of the operators to repeat for ongoing monitoring purposes, and unlikely that an accredited laboratory could do it without it becoming prohibitively expensive.

I.1.9. The fact that spore strip tests are proven to give equivalent results to the spore suspension tests means that these could be used for routine ongoing monitoring. Spore strip tests are much easier for the operators to perform, and as the strips are sealed in a glassine envelope, which removes many of the technical difficulties and dangers of contamination, these tests are much to be preferred.

I.1.10. It is my recommendation that spore strip tests be used to monitor the efficacy of the MetaMizer system for regular testing purposes.

I.2. THERMAL INTEGRATOR RESULTS

1.2.1 No thermal integrator strips were available that tested temperatures of 139° to 142°C for 4 minutes, so strips that tested parameters close to this were used. These tested conditions of 134°C for 4, 5 or 11 minutes respectively, or the equivalent.

1.2.2. When tested, all these strips changed colour indicating that, at the very minimum, the above conditions had been met. It is likely, given the MetaMizer manufacturers' information that the system runs between 139° and 142°C for 4 minutes that these conditions were exceeded. It was not possible to measure this accurately with the strips available.

I.3. AIR SAMPLING

I.3.1. The results of the air sampling show that the environment in and around the plant is free of any pathogenic micro-organisms and coliform organisms. Low levels of environmental and skin micro-organisms were found, which are to be expected wherever there is occupation and movement.

I.3.2. The RCS air sampler employed in the air sampling operates on a similar principle to a slit sampler, in that measured volumes of air are drawn into the sampler and actively impacted onto agar, providing a viable count. The air is drawn into the sampler by centrifugal action, rather than by vacuum as in the slit sampler. This sampler has an accuracy of ±2% (Figure 4).

I.3.3. Centrifugal samplers are recommended as the most suitable method for monitoring disperse releases of micro-organisms by air at clinical waste sites in Environment Agency guidelines[1].

I.3.4. The RCS sampler was used in preference to a Cassella slit sampler in these studies as it is smaller and more portable, whilst still providing comparable results.

I.3.5. The Environment Agency have published 'trigger levels' for bioaerosols at clinical waste transfer stations based on custom and practice[7], which must be related to existing background levels. The levels detected here can therefore be used as background levels, as they were obtained before any clinical waste was processed in the plant.

I.3.6. Trigger levels of >1000 cfu of bacteria and fungi, and >300 cfu of Gram-negative bacteria have been suggested[7]. Similar levels have also been suggested by the American Industrial Hygiene Association[8]

J) CONCLUSIONS

J.1. The operation of the Medivac MetaMizer Clinical Waste Treatment units was observed to be compatible with the stated practice, and the operational parameters were stated as139° to 142°C for a holding time of 4 minutes.

J.2. At these parameters, the MetaMizer proved capable of achieving a >6 $\log_{10}$ kill of spores of *B atrophaeus (subtilis)* reproducibly over 5 full cycles over a testing period of 2 days. This was confirmed using two separate testing protocols based on the Environment Agency's technical guidance Appendix A and Appendix B.

J.3. Microbiological air testing at designated points in and around the plant showed that the level of airborne contamination was well inside Environment Agency trigger levels. No pathogenic microorganisms were found.

J.4. The clinical waste treatment plant is therefore proven to be microbiologically effective to greater than the required level, and background levels of microbial contamination have been established as a benchmark for future monitoring.

J.5. It is my opinion that the plant can safely, effectively and reproducibly treat clinical waste to make it safe for final disposal.

REFERENCES

1) Environment Agency. Technical Guidance on Clinical Waste Facilities. Version 2.5. July 2003

2) Clinical Waste Disposal/treatment technologies (alternatives to incineration) Health Technical Memorandum 2075. NHS Estates. 1998

3) Technical Assistance Manual: State Regulatory Oversight of Medical Waste Treatment Technologies. A Report of the State and Territorial Association on Alternate Treatment Technologies. April 1994.

4) Slavik,NS. 1995. Use of Germicides in Medical Waste Treatment. In: Chemical Germicides in Health Care. Ed. Rutala WA. Association of Professionals in Infection Control and Epidemiology, Inc. ISBN 0 921317-48-4. p 288-299

5) Holliday MG, Ford M, Burrell P, Gould FK. 2000. Heat disinfection of clinical waste: microbiological assessment and monitoring of effectiveness. *British Journal of Biomedical Science.* **57**: 107-113

6) Biological Testing of a Clinical Waste Treatment Device Manufactured by Medivac Technology pty Ltd. 2002. Report prepared by Silliker Microtech pty Ltd. (Supplied by Cannon Hygiene)

7) Environment Agency. Technical Guidance Document (Monitoring) M17. Monitoring of Particulate Matter in Ambient Air around Waste Facilities. March 2003.

8) Aerobiological Engineering. Limits for Indoor Airborne Microbes. http://www.engr.psu.edu/www/dept/arc/server/wjk/purge.htm

FIGURE 1 MEDIVAC METAMIZER SYSTEM

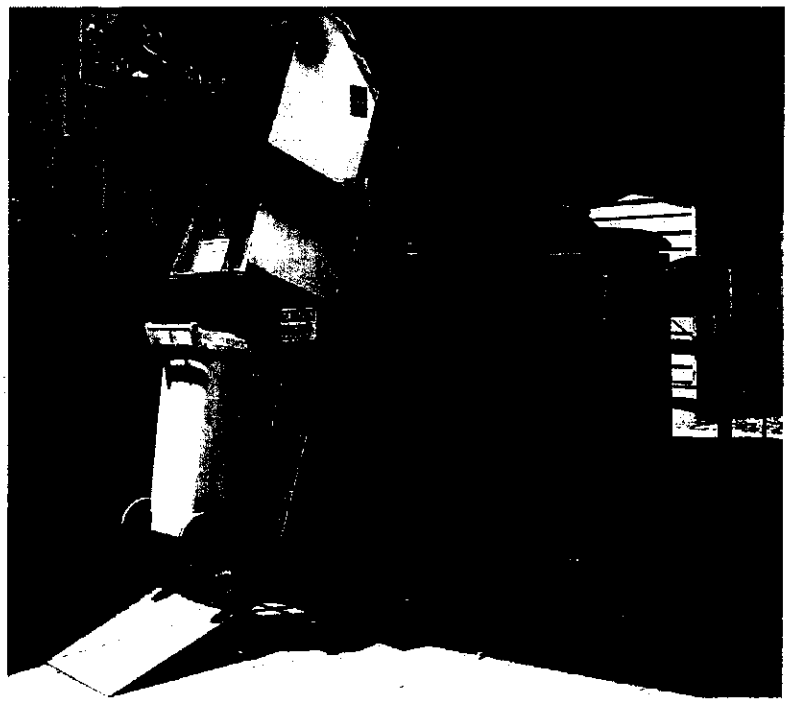


FIGURE 2. NET BAGS USED AS SPORE CONTAINERS

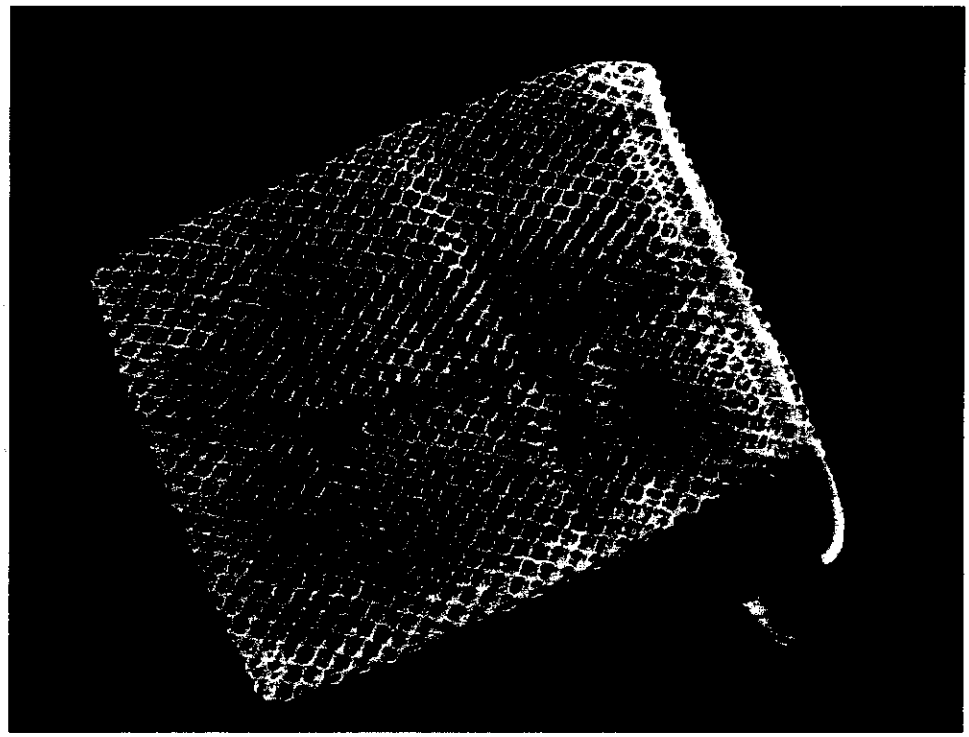


FIGURE 3 SITES OF AIR SAMPLES, CANNON HYGIENE, NORTHGATE WHITE LUND

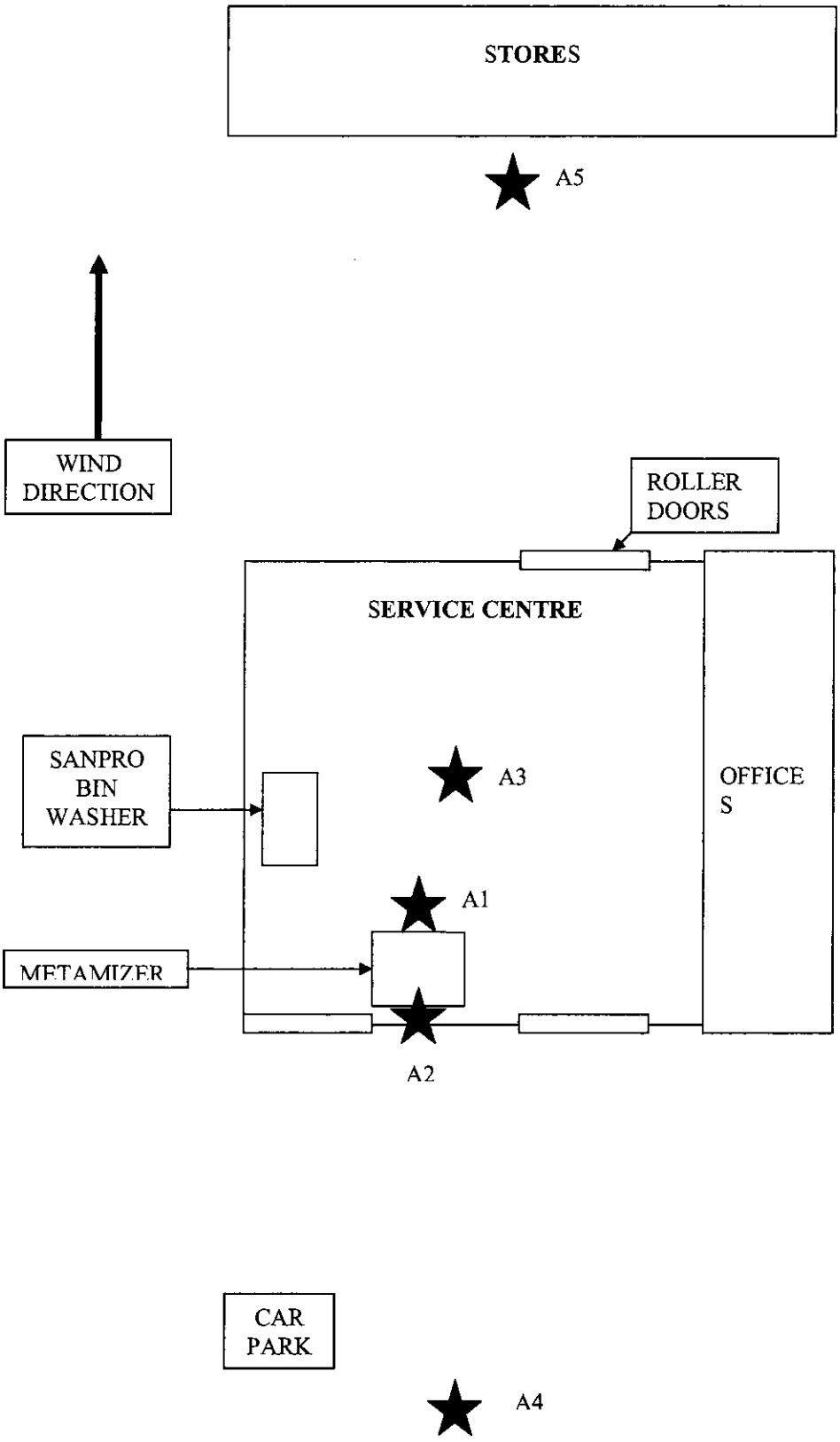
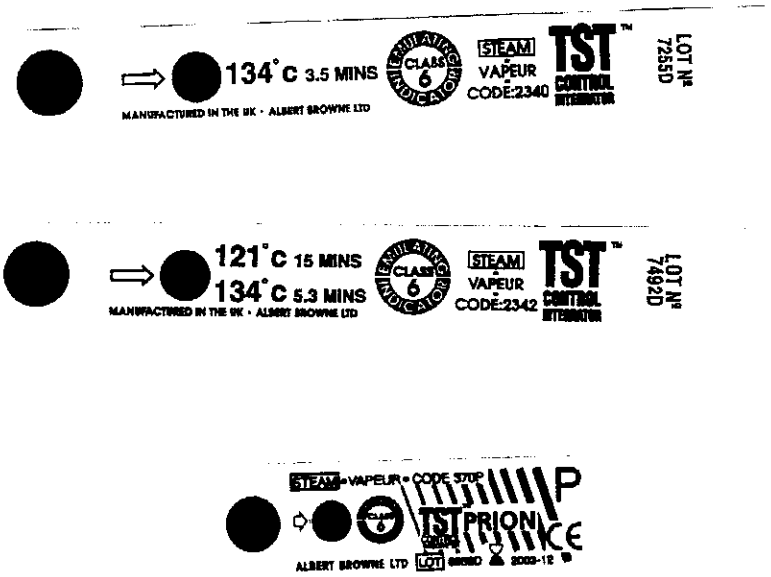


FIGURE 4. RCS AIR SAMPLER

FIGURE 5 THERMAL INTEGRATOR STRIPS AFTER EXPOSURE



APPENDIX 1
QUALITY DOCUMENTATION FOR SPORE STRIPS

RAVEN BIOLOGICAL LABORATORIES, INC.
P.O. BOX 27261
8607 PARK DRIVE
OMAHA, NEBRASKA 68127
TELEPHONE: (402) 593-0781
1-800-728-5702
FAX ADMIN: (402) 593-0921
ORDER PROCESSING FAX: (402) 593-0995
EMAIL: qa-ra@ravenlabs.com
www.ravenlabs.com

CERTIFICATE OF ANALYSIS
RAVEN BIOLOGICAL INDICATORS

Bacillus atrophaeus' Spore Strips - Recommended for use in evaluating Dry Heat or Ethylene Oxide gas sterilization processes.

This document certifies that the biological indicators for this lot meet Raven Biological Laboratories' quality control specifications, ISO 11138 parts 1 & 2, EN 866 parts 1 & 2, and suggested performance parameters published in the current United States Pharmacopeia.

Wendy Royalty-Hann
Quality Assurance Manager
Raven Biological Laboratories, Inc.

Release: **04/06/2004**

Performance Data for Lot # **1162322** Batch **232GB** Expiration Date **4/06**

Organism: ***Bacillus atrophaeus***' ATCC No. 9372

Mean Strip Recovery	1.5 x 10 *	CFU* /1.5" x 0.25" strip
D_{60} Value**	3.6	minutes (600 mg EtO/L, 54°C, 60% RH- This accuracy shall not exceed +/- 0.5)
D_{160} Value**	2.5	minutes (Dry Heat, 160°C- This accuracy shall not exceed +/- 0.2)
Z-value***	39.0	°C; approximate (based on EN 866-6.)

* Colony Forming Units
** Determined at time of manufacture, Spearman-Karber method. The D-value is reproducible only under the exact conditions under which it was determined. The user would not necessarily obtain the same results. Therefore, the user would need to determine the suitability for its particular use.
*** See reverse side
' Strain derived from ATCC# 9372 has been reclassified and is now called *Bacillus atrophaeus* (formerly *Bacillus subtilis*)

Resistance Characteristics: (Based on US Pharmacopeia Calculations)

AGENT	CONDITIONS	SURVIVES	KILLED
Ethylene Oxide	54 ± 1°C, 600 ± 30 mg/L, 60 ± 10% RH	15.0 min.	36.6 min.
Dry Heat	160 ± 2°C	10.4 min.	25.4 min.

Purity: No evidence of contaminants using standard plate count techniques.
Incubation: 7 days in soybean-casein digest broth at a temperature of 30 - 35°C
Storage: 15 - 27°C (60 - 80°F). 30 - 70% RH, away from sterilizing agents, direct sunlight and all other forms of UV light. (Do Not Refrigerate).
Disposal: Do not use after expiration date. Sterilize all cultures before discarding.

2/04/03

APPENDIX 2
QUALITY DOCUMENTATION FOR SPORE SUSPENSION

RAVEN BIOLOGICAL LABORATORIES, INC.
P.O. BOX 27261
8607 PARK DRIVE
OMAHA, NEBRASKA 68127
TELEPHONE: (402) 593-0781
1-800-728-5702
FAX ADMIN: (402) 593-0921
ORDER PROCESSING FAX: (402) 593-0995
EMAIL: qa-ra@ravenlabs.com
www.ravenlabs.com

CERTIFICATE OF ANALYSIS

RAVEN BIOLOGICAL INDICATORS

Bacillus atrophaeus[1] Spore Suspension - Recommended for use in evaluating **ethylene oxide gas** and **dry heat** sterilization processes.

This document certifies that the biological indicators for this lot meet Raven Biological Laboratories' quality control specifications, EN 866-2 and 866-6, and suggested performance parameters published in the current United States Pharmacopeia.

Wendy Royalty-Hann/TH
Wendy Royalty-Hann
Quality Assurance Manager
Raven Biological Laboratories, Inc.

Release: **05/20/2004**

Performance Data for Lot # **1082331** Batch **233GB** Expiration Date **5/05**

Organism: ***Bacillus atrophaeus***[1] ATCC No. 9372

Volume **10** ml 40% Ethanol in DI water suspension / vial

Label Population Claim **2.8** x 10^{6} CFU* / 0.1 ml

Mean Suspension Recovery **2.8** x 10^{6} CFU* /ml

D_{160} Value** **2.6** minutes (Dry Heat at 160 C)

D_{EO} Value** **3.4** minutes (600 mg/L EtO, 54 C, 60% RH)

* colony forming units

** Determined at time of manufacture on paper strip carrier, using Fraction Negative analysis (Spearman-Kaerber method)

[1] The D-value is reproducible only under the exact conditions under which it was determined. The user would not necessarily obtain the same results. Therefore, the user would need to determine the suitability for its particular use.

[1] Strain derived from ATCC# 9372 has been reclassified and is now called *B. atrophaeus* (formerly *Bacillus subtilis*).

Resistance Characteristics:

Survival time (in minutes) = not less than (labeled D-value) x ($\log_{10}$ labeled spore count per carrier - 2); and Kill time (in minutes) = not more than (labeled D-value) x ($\log_{10}$ labeled spore count per carrier + 4).

Purity: No evidence of contaminants using standard plate count techniques.

Incubation: 7 days in soybean-casein digest broth at a temperature of 30 - 35°C

Storage: Refrigerate at 2 to 8 C.

Disposal: Do not use after expiration date. Sterilize all cultures before discarding.

2/04/03

APPENDIX 3 CALCULATIONS FOR THE EVALUATION OF SPORE SUSPENSION CHALLENGE TESTS

From the number of organisms counted on the test and control plates, the number of viable spores recovered per run can be calculated and the $\log_{10}$ reduction in viable spore numbers can be calculated for each cycle.

a) Initial inoculum of spores per gram of waste. (IC)
Inoculate 10^{10} spores in 7 kg of waste
= 1.4×10^{6} spores per gram

b) Recovery of spores from Control run. (RC)
Number of spores counted per plate x dilution factor
= Number of spores recovered per gram

c) Recovery of spores in the Test run. (RT)
Number of spores counted per plate x dilution factor
= Number of spores recovered per gram

d) Calculation of $\log_{10}$ reduction

Control results : $\log_{10}RC = \log_{10}IC - \log_{10}NR$

$\log_{10}RC = \log_{10}$(number of spores/gram recovered)
$\log_{10}IC = \log_{10}$(number of spores/gram inoculated)
$\log_{10}NR = \log_{10}IC - \log_{10}RC$

Test results : $\log_{10}IC - \log_{10}NR - \log_{10}RT$
$\log_{10}RT = \log_{10}$(number of spores/gram recovered)

$Log_{10}Kill =$ $\log_{10}IC - \log_{10}NR - \log_{10}RT$