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TECHNICAL MANUSCRIPT 245

DISINFECTION
OF A HOSPITAL OPERATING ROOM
AND 5-BED WARD
WITH BETA-PROPIOLACTONE

Manuel S. Barbeito

OCTOBER 1965

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DISINFECTION OF A HOSPITAL OPERATING ROOM AND 5-BED WARD
WITH BETA-PROPIOLACTONE

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DIRECTORATE OF INDUSTRIAL HEALTH AND SAFETY

Project 1C622401A072

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ABSTRACT

Beta-propiolactone was used to disinfect a community hospital ward and operating room following their potential contamination with clostridial organisms. A description of the hospital’s physical facilities and the methods employed to accomplish the disinfection are reported.
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I. INTRODUCTION

Postoperative examination of the leg stump of a male patient in a five-bed ward at Frederick Memorial Hospital revealed possible infection with one or several of the organisms that produce gas gangrene. During examination of the wound by the attending physician, flowing pus contaminated the bed clothes and the asphalt-tiled floor. Secondary contamination of surfaces probably was caused by the liberated bacterial aerosol. Subsequently, the wound was dressed and the patient prepared for surgery. Other patients in the ward at the time the wound was examined were transferred to other locations throughout the hospital.

Once the tentative diagnosis was made that the patient had gas gangrene, the ward was declared an isolation area. The pillows in the ward were placed in plastic bags, which were then sealed, removed from the ward, transported to an autoclave and sterilized with ethylene oxide. After placing the pillows in the autoclave equipped for use with ethylene oxide, the plastic bags were punctured and ethylene oxide was added to the autoclave in the prescribed manner. The pillows remained in the autoclave for 18 hours, and were then aerated for a minimum of 48 hours before being used. Because the mattresses were protected with sheets and an impermeable protective covering, it was decided that the sheets would be removed and handled in the manner prescribed for disposal of contaminated linens. The mattresses with a plasticized covering were scrubbed with a Tergisyl solution, removed from the ward, and placed in the sunlight for 8 hours.

Following reamputation and time in the postoperative room the male patient was transferred to another room within the hospital.

Upon completion of surgery, the operating room was isolated and the equipment remained within the room. The operating room staff entered the room with protective operating room clothing and wiped all surfaces and equipment with toweling soaked in a solution of Tergisyl.

At a meeting of the Laboratory Infections Committee of the Frederick Memorial Hospital, it was decided to ask the assistance of Industrial Health and Safety Division, Fort Detrick, in regard to a practical and safe method by which to disinfect the operating room and ward. Examination of the facilities by Fort Detrick personnel revealed that the most feasible method to accomplish disinfection was through the use of beta-propiolactone (BPL) as a vapor. In addition it was concluded that the

* Source - Lehn & Fink. Active ingredients - orthohydroxydiphenyl, paratertiary amylphenol, sodium sulfonates.
** Disinfect as used in this report means that the chemical agent BPL acted as a bactericide and sporicide.
disinfection would be done after all scheduled surgery for the day was completed to eliminate the possibility of having BPL vapors enter the other operating rooms during an operation, either through leakage within the building structure or through the exhaust supply ventilation system. Another factor considered before disinfection with BPL was the wind direction and general atmospheric conditions. The weather on the evening when the operating room was disinfected was clear and warm with a southerly wind that carried the vapors away from the air supply duct. The afternoon when the ward was disinfected also was warm and clear with a westerly wind. The ward was disinfected in the afternoon because the possible problems associated with entry of BPL into other areas were far less than if BPL were to enter the operating rooms.

Because the normal flora of the facilities to be disinfected were not known, it was decided that spores of Bacillus subtilis var. niger would be used as a bacterial tracer to determine the efficacy of the disinfection. By personal experience and extrapolation it was concluded that if B. subtilis and Bacillus anthracis spores are killed with BPL, both the vegetative and/or spore states of clostridia organisms would be killed.
II. DESCRIPTION OF FACILITIES

A. OPERATING ROOM

The operating suite is located on the top (third) floor of the hospital at the far end of one wing. The walls and area around the window of the operating room (Fig. 1) used for surgery on the gas gangrene wound case are ceramic-tiled up to a height of 7 feet 1 1/2 inches. The remainder of the walls and ceiling are plastered and covered with an oil-base paint. The floor covering is resilient conductive rubber. Solid wood flush doors are used in the operating room. Windows are located along the outside wall. A double scrub sink is located in one corner of the room and a recessed instrument storage cabinet is along one wall (Fig. 1).

One exhaust fan mounted 36 inches above the roof evacuates the air from the three operating rooms. The exhaust duct from each operating room empties into a common manifold, which has four 180° elbows that direct the air downward towards the roof. Each operating room is equipped with its separate air conditioner that is located in the stairwell. The air conditioner is thermostatically controlled (both temperature and relative humidity) from within the operating room. The supply fan switch is located in the operating suite hallway. One air supply louver (size 24 inches x 24 inches), 30 inches above the roof level on the vertical stairwell wall 19 feet away from the exhaust duct, serves the three air conditioners. The outside air passes through an "A" frame expanded aluminum mesh washable dust stop filter that is within the air conditioner. The ventilation system is 100% make-up air.

The volume of the operating room is approximately 3,738 cubic feet.

Air enters the operating room at the ceiling through a centrally located diffuser (18 inches x 18 inches) and is exhausted through a duct opening (20 inches x 20 inches) in a side wall mounted 2 inches above the floor (Fig. 1). A void space made with cinder blocks within the building's walls serves as the exhaust duct. It is connected to a metal manifold duct before it enters the exhaust blower housing on the roof.

Equipment in the operating room during surgery and disinfection was (i) face masks for use with anesthetizing gases; (ii) anesthesia machine with associated gas cylinders and hoses; (iii) stainless steel Mayo and instrument stands, stools, and kick buckets; (iv) sealed jars of intravenous fluids; (v) wooden arm boards with a rubber-covered sponge padding; (vi) canvas restraining straps; (vii) standard operating room lights, and (viii) a suction machine used for aspirations.
Figure 1. Schematic Drawing of Operating Room, Frederick Memorial Hospital.
B. WARD

The five-bed male ward is located approximately midway along the main corridor on the second floor (Fig. 2). The ward is adjacent on one side to an outside porch that is directly above the main entrance and offices of the hospital, on the second side to a women's five-bed ward, and on the third side to a service room. The X-ray department is located above the ward on the third floor. The ward has two windows, one of which is equipped with 2-foot high double doors below the window and extending to the floor to permit entrance onto the porch when both the doors and window are opened. The other window has an air conditioner installed in the lower part of the window. Two 8-inch oscillating fans are located on opposite walls 7½ feet above the floor. The entrance-exit door is centrally located along the corridor wall.

The walls and ceiling of the ward are plastered and covered with an oil-base paint, and the floor is covered with asphalt tile. The volume of the ward is approximately 3,250 cubic feet.

Equipment within the ward during disinfection with 3% was (i) five metal bed frames with springs, (ii) five metal bed stands with their normal contents, (iii) two wooden chairs, (iv) five metal foot stools with rubber treads, and (v) three plastic waste buckets.
Figure 2. Schematic Drawing of 5-Bed Ward, Frederick Memorial Hospital.
III. DISINFECTION

A. OPERATING ROOM

The operating room (Fig. 1) was prepared for disinfection by (i) elevating the ambient room temperature to 75°F; (ii) raising the relative humidity to 75% by injecting steam into the air supply system duct after the "A" frame filter; (iii) taping the peripheral seams of the doors with pressure-sensitive tape; (iv) turning off the air supply fan; (v) sealing shut the door of the surgical instrument supply cabinet with pressure-sensitive tape; and (vi) covering both the inlet supply duct on the outside stairwell wall and the exhaust duct within the room with polyethylene film. This film was attached to the walls around the exhaust duct lower with pressure-sensitive tape. The film covering the exhaust duct lower within the operating room was equipped with a twin tear strip; a heavy piece of twine extended to the adjoining hallway to provide a means of removing the polyethylene film cover without anyone's having to enter the BPL-laden room after the BPL contact time was complete. A piece of absorbent paper was placed beneath the generator to prevent possible damage to the floor during disinfection of the BPL.

Before disseminating the BPL 12 clearly delimited sites throughout the operating room were seeded with spores of Bacillus subtilis var. alger (count $1 \times 10^6$ spores per milliliter) by dipping a cotton swab into the liquid culture and transferring the spores onto the selected surfaces. Areas contaminated with the spores were wall, window sill and floor surfaces, scrub sink and soap dish, door handle, operating table, stand, and anesthetizing machine. The purpose of seeding the areas with spores was to determine the efficacy of the disinfection procedures.

Nine hundred milliliters of BPL were disseminated (amount calculated at a rate of one gallon for each 12,000 to 16,000 cubic feet of space) with a Challenger generator, Model 5100 CR. (See Figure 1 for generator location.) Following dissemination and a 2-hour contact period the polyethylene films were removed, first from the exhaust lower by means of the twin run under the door into the corridor, and then from the supply lower. Then the exhaust fan was turned on. Air was exhausted for 30 minutes under this arrangement, which kept the operating room under a slight negative pressure and allowed fresh air to enter the room through the supply system. The supply fan was started 2½ hours after termination of BPL dissemination. With both the supply and exhaust fans on, the operating room was under a slight positive pressure, but because of the two-inch-wide pressure-sensitive tape sealing all cracks around the doors, the vapors of BPL did not enter the adjoining hallway or service room. After 2½ hours of aeration a portion of the tape was removed from one door to permit sampling of the air for the detection of residual BPL vapors. Approximately 100 cc of air from within the operating room was drawn through a chemical agent detection tube that will respond to
BEL concentrations lower than 0.01 milligram per liter. The test is based on the appearance within the tube of a blue color caused by the reaction of BEL with the p-nitrobenzyl pyridine absorbed on silica gel. The sensory system of man himself will detect 0.05 milligram of BEL per liter of air.

The twelve previously seeded areas were sampled with sterile cotton swabs moistened with physiological saline for the recovery of B. subtilis. The cotton swabs were streaked on corn steep agar (see Appendix) in plastic petri dishes and incubated at 37°C for 48 hours. A duplicate set of samples was taken of the seeded areas with sterile swabs moistened with distilled water. Swabs were aseptically transferred to thioglycolate broth tubes and incubated at 37°C for 24 hours. Controls were used in conjunction with the test samples and were satisfactory. No B. subtilis or other microorganisms were recovered with the cotton swabs. However, the surface sample from the window sill that was inoculated into a thioglycolate broth tube showed turbidity. The thioglycolate broth tube inoculated with B. subtilis as a control also showed a comparative amount of turbidity. Microscopic examination with Gram stain of both the control and test sample revealed gram-positive rods. Subsequent bacteriological examination and testing revealed that the recovered microorganism from the ceramic window sill was Bacillus cereus. One of the possible sources for B. cereus contamination following disinfection is contamination of the broth tube, or swab, or entrance of the organism into the operating room through the ventilation system.

To determine if air leaked around the door's seams a BSA ventilation smoke tube was used. It also was used to determine the direction of air currents from the exhaust duct once aeration of the operating room commenced. Air leakage and current tests were run before, during, and following dissemination of the BEL to preclude difficulties with penetration of the BEL vapors into other occupied areas within the hospital.

An unforeseen emergency surgical operation was begun in the operating room adjacent to the service room that also served the operating room being disinfected, at the same time that the supply fan was turned on (Fig. 1). Because of the wind direction and atmospheric conditions, vapors of BEL did not enter the ventilation supply system to the operating suite, so that the emergency operation proceeded without incident.

Subsequently, all surfaces within the disinfected operating room and its equipment were wiped down with cloths moistened with Tergisyl solution.

* Falcon Plastics Division of B-D Laboratories, Inc., 5500 West 83rd St., Los Angeles, California, 90045.
B. WARD

The relative humidity within the ward was raised by spraying tepid water from a Challenger generator until 77% was obtained throughout the ward. The ambient temperature was 74°F.

Before BPL was disseminated, 16 carefully delimited surfaces within the ward were seeded with *B. subtilis* var. *niger* spores in the same manner as in the operating room. Areas seeded were door knob, beds, chair, stool, floor and wall surfaces, bed stand, curtain rod, waste basket, window sill, and foot stool.

The oscillating fan opposite the generator location (Fig. 2) was turned on low speed to help distribute the BPL vapors throughout the ward with some degree of homogeneity.

Cracks on the windows were taped with pressure-sensitive tape. The window with the double doors beneath, serving as a doorway to the porch, was arranged so that later both it and the doors could be opened from the outside. Windows to patients' rooms along the second floor corridor were closed to preclude entrance of BPL vapors into the rooms during the phases of BPL disinfection. All doors and drawers of the metal bed stands were opened. The generator containing the BPL was positioned so that BPL vapors would not impinge directly on any surface; in addition, a piece of absorbent paper was placed beneath and approximately 8 feet in front of the generator. From personal experience we have learned that asphalt tile is dissolved if liquid droplets of undiluted BPL fall directly onto the tile. After the Challenger generator was turned on by person who then immediately left the ward, the peripheral cracks of the door were taped with pressure-sensitive tape.

Smoke tubes were used to determine if any air was leaking from within the ward into the corridor during dissemination of the BPL, during the contact period, or during aeration.

A two-hour contact period was maintained after the BPL was disseminated. Then the double doors beneath the one window were opened from the outside and a 20-inch exhaust fan was placed in the opening. The BPL vapors were exhausted from the room with the fan positioned so that the created air stream directed the BPL-laden air away from patients' rooms. During the initial aeration period, personnel monitored the patients' rooms for penetration of BPL vapors.

After two hours' aeration, personnel could enter the ward without chemical respiratory protective masks. The level of BPL vapors at that time was below the detectable range of the Civil Defense detection tubes. However, to insure that all residual BPL vapors and breakdown products
were removed from the ward, the other oscillating wall-mounted fan and the window air conditioner fan were turned on and allowed to run overnight, in addition to the window exhaust fan. The tape was removed from the door after the two-hour aeration period to permit a greater volume of air to be drawn from the hallway through the openings around the door. With the above arrangement of fans the ward was under slight negative pressure during the entire aeration period. This prevented vapors of BPL from entering adjoining areas.

After the initial two-hour aeration, sterile cotton swabs moistened with physiological saline were used to sample the previously seeded areas for the recovery of *B. subtilis*. Upon return to the microbiological laboratory, the cotton swabs were aseptically streaked on corn meal agar (see Appendix) in plastic petri dishes. The plates were incubated at 37°C for 48 hours. No *B. subtilis* or other microorganisms were recovered after disinfection of the ward with BPL. This showed that a satisfactory disinfection was achieved. Subsequently, the ward and contents were wiped with water-moistened cloths.
IV. CONCLUSIONS AND DISCUSSION

A hospital ward and an operating room were disinfected by disseminating beta-propiolactone (BPL) in a concentration of one gallon of BPL for each 12,000 to 16,000 cubic feet of space. The temperature was elevated to 74°F and the relative humidity to 75%, the supply and exhaust ventilation systems were turned off, and all seams and openings were blocked before BPL was disseminated. It was possible to enter the disinfected areas after two hours of forced aeration. However, overnight aeration is advisable whenever BPL is used.

The efficacy of BPL disinfection was determined by seeding areas with spores of Bacillus subtilis var. niger (count \(1 \times 10^6\) spores per mL).

Critical visual examination of the operating room revealed no apparent damage to items occasionally affected by liquid BPL, such as the painted surfaces, synthetic rubber products, or plastic items. In the ward the only apparent damage was to the varnished door. It is the opinion of the writer, after examining other doors of the same age (approximately 25 years old) throughout the hospital, that any elevation of humidity would have caused the slight blistering of the varnish that was evident on the door to the ward disinfected with BPL.


**Appendix**

**Corn Steep Liquor Medium**

Stock Solution - Dissolve 500 grams of crude black strap molasses in 1250 milliliters of corn steep liquor.

<table>
<thead>
<tr>
<th>Medium:</th>
<th>Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molasses and corn steep stock solution</td>
<td>31 ml</td>
</tr>
<tr>
<td>Bacto-agar</td>
<td>20 grams</td>
</tr>
<tr>
<td>Distilled H₂O</td>
<td>1000 ml</td>
</tr>
<tr>
<td>5 N NaOH</td>
<td>0.75 ml</td>
</tr>
<tr>
<td>Actidione (dry)</td>
<td>10 mg</td>
</tr>
</tbody>
</table>

Preparation - Dissolve agar in water completely, add stock solution, actidione, adjust pH, then autoclave. Final pH to be 6.8 - 7.2.
Beta-propiolactone was used to disinfect a community hospital ward and operating room following their potential contamination with clostridial organisms. A description of the hospital's physical facilities and the methods employed to accomplish the disinfection are reported.