J. Hyg., Camb. (1968), **66**, 625
With 2 plates
Printed in Great Britain

Investigation and treatment of floors of patients' rooms: a study with an agar cylinder

By J. S. KUIPERS

Bacteriological-Serological Laboratory, University of Groningen (Netherlands)

(*Received* 30 *March* 1968)

INTRODUCTION

Nearly everywhere in patients' rooms bacteria can be isolated. Besides the air and textiles, the principal centres are smooth surfaces, such as floors, window-sills and similar places.

The flora found in these rooms often reflects not only that found on patients admitted, but also on personnel and visitors.

It is necessary to have at one's disposal a method which not only can ascertain contaminations accurately and at an early stage, but which can also determine the degree of contamination. The latter point is especially important in judging the effect of certain cleaning and disinfection methods.

Most investigators are satisfied, when testing, with a qualitative examination and use sterile cotton-wool containers moistened with broth (Noble & Lidwell, 1963). This method is very easy to apply, but gives little information on the number of bacteria found per unit of surface area. Examination of swept dust or dust from a vacuum cleaner is also easily carried out. However, this does not give quantitative data either. When sweeping, we do not collect the smallest particles, because these are distributed by this action through the air and keep floating there for a long time (Cruickshank, 1965). The smallest particles in particular, with a diameter of less than $2\,\mu$, most often cause infection upon inhalation (Riley & O'Grady, 1961). The 'impinger method' used by Williams (1949) also only yields qualitative data.

Foster (1960) used 'impression plates' for the examination of floors. Gentles (1956) applied a replica technique. With the help of these methods both investigators were able to ascertain accurately the quantitative contamination of various parts of the floors of patients' rooms. However, both methods are rather complicated. Also, the 'agar sausage method' described by Ten Cate (1965) has its disadvantages, because the preparation of the sausages is difficult and time-consuming.

We have tried to find a method which is easy to apply, with which good results could be obtained qualitatively as well as quantitatively, and which could be used to compare the various cleaning and disinfecting methods and test their value. To this end we have devised an agar cylinder.

METHODS

Plate 1A shows the cylinder with accessories. The cylinder is closed with a lid. It, as well as the knife and sheath, is made of stainless steel and can easily be sterilized in an autoclave.

The agar cylinder is easy to handle. After sterilization the cylinder can be filled with various media, depending on the investigation we wish to carry out. Besides broth-agar we can use blood-agar or the medium of Chapman (1946) to isolate *Staphylococcus aureus*, glycerol-agar* for the isolation of *Pseudomonas aeruginosa*, while for the isolation of Gram-negative rods endo-agar can be used. In order to prevent the media from sliding out of the cylinder during the stamping, we have always used media prepared with 1·8 % Bacto-agar which were 1 day old. After the lid has been removed the medium is pushed 0·5 cm. out of the cylinder by turning the piston 2 or 3 strokes (Pl. 1 B). The medium is then pressed against the floor which is to be examined (Pl. 1 C). As the cylinder does not touch the floor, there is little chance of its being contaminated. With the knife a 0·5 cm. thick slice is cut off (Pl. 1 D) and placed in a Petri dish (Pl. 2 A). With one filled cyclinder about 20 tests can be done. The slices in the Petri dishes will be examined in the usual way after incubation.

RESULTS

An investigation has been carried out on the contamination of the floor with *Pseudomonas aeruginosa* in a patient's room at the respiratory centre in Groningen. This organism had been isolated at some time from the tracheobronchial aspirate of all four patients in this room. The examination of the floor with the cylinder filled with glycerol-agar showed that nearly all parts of the floor were contaminated with *Ps. aeruginosa* (Fig. 1). The contamination was especially high near the openings of the exhausts of the four Fricar suction pumps, which were at that time about 10 cm. from the floor. At the time of this investigation the floors were cleaned daily with soap and water. This treatment probably not only helped to spread the micro-organisms, but also gave them a chance to multiply. Thus we found fewer bacteria per surface unit immediately after treatment of the floor than 10 hr. later (Pl. 2 B, C). *Ps. aeruginosa* could not be isolated, after treatment of the floor with 0·3 % Halamid, for more than 12½ min.

To investigate the effect of daily sweeping only, a floor was sprayed with 1 ml./m.^{2} of a diluted broth culture containing 2×10^4 *Ps. aeruginosa* and $2{\cdot}3 \times 10^4$ *Staph. aureus* per ml. The floor was cleaned daily with a sterilized sweeper. Cylinders containing glycerol-agar and blood-agar were used for the isolation of the two organisms. The results of daily testing are shown in Table 1.

In Table 2 the results are given for a floor cleaned with water and a strongly alkaline soap (pH 9·5). This floor was sprayed with 1 ml./m.^{2} of a diluted broth

* The composition of the glycerol-agar used is as follows: 1 % (w/v) glycerol (= 0·81 % v/v), ½ % Difco proteosepepton, 0·04 % K_2HPO_4, 2 % $MgSO_4.7H_2O$, 0·001 % $FeSO_4$ (= 0·003% $FeSO_4.7H_2O$), pH 7·2–7·1. Add 1·8 % Bacto-agar and sterilize during 15 min. at a temperature of 120° C.

culture containing 2×10^4 *Ps. aeruginosa*, $2{\cdot}4 \times 10^4$ *Alcaligenes faecalis* and $2{\cdot}3 \times 10^4$ *Staph. aureus* per ml. Cylinders containing glycerol-agar, endo-agar and blood-agar were used for testing. The experiment was ended 150 min. after the floor was cleaned, at which time all three organisms could still be isolated.

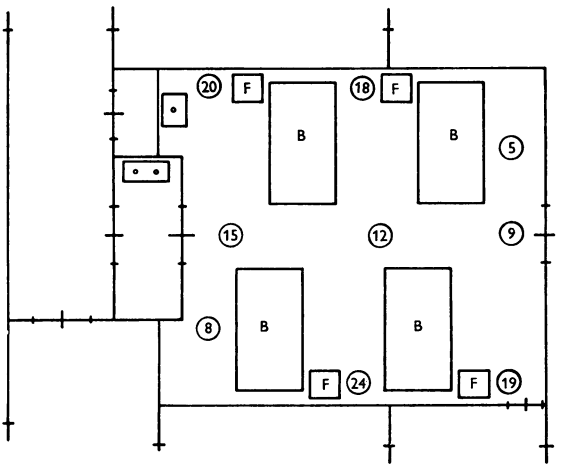


Fig. 1. The number of *Pseudomonas aeruginosa* colonies per print in various places in a room, into which four patients infected with *Ps. aeruginosa* had been admitted. B = bed, F = Fricar suction-pump.

Table 1. *Results of daily cultures from a floor that was swept daily for* 17 *days after infection*

Days after infection	Isolation of *Pseudomonas aeruginosa*	Isolation of *Staphylococcus aureus*	Days after infection	Isolation of *Pseudomonas aeruginosa*	Isolation of *Staphylococcus aureus*
1	+	+	10	+	+
2	+	+	11	+	+
3	+	+	12	+	+
4	+	+	13	+	+
5	+	+	14	+	−
6	+	+	15	+	−
7	+	+	16	−	−
8	+	+	17	−	−
9	+	+			

+ = growth; − = no growth.

In Tables 3–5 the results are given of experiments with floors cleaned with soap and water and then treated with one of three disinfectants, 0·3 % Halamid (chloramine T), 2 % liquor cresoli saponatus, or 2 % Lyorthol. The infecting organisms and the method of testing were the same as those used in the previous experiment. To neutralize the disinfectants, 1 % sodium thiosulphate was added to the medium in the experiments with Halamid, and 1 % Tween 80 in the experiments with liquor cresoli saponatus or Lyorthol (Williams *et al.* 1966).

Table 2. *Results of cultures from a floor cleaned with water and a strongly alkaline soap, after infection*

Time after cleaning (min.)	Isolation of *Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Staphylococcus aureus*
10	+	+	+
20	+	+	+
30	+	+	+
40	+	+	+
50	+	+	+
60	+	+	+
70	+	+	+
80	+	+	+
90	+	+	+
100	+	+	+
110	+	+	+
120	+	+	+
130	+	+	+
140	+	+	+
150	+	+	+

+ = growth.

Table 3. *Results of cultures from a floor cleaned with soap and water and disinfected with* 0·3 % *Halamid after infection*

Time after disinfection (min.)	Isolation of *Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Staphylococcus aureus*
1	+	+	−
2½	+	−	−
5	+	−	−
7½	+	−	−
10	+	−	−
12½	−	−	−
15	−	−	−

+ = growth; − = no growth.

Table 4. *Results of cultures from a floor cleaned with soap and water and disinfected with* 2 % *liquor cresoli saponatus after infection*

Time after disinfection (min.)	Isolation of *Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Staphylococcus aureus*
1	+	+	+
2½	+	+	+
5	+	−	+
7½	+	−	−
10	−	−	−
12½	−	−	−
15	−	−	−

+ = growth; − = no growth.

Table 5. *Results of cultures from a floor cleaned with soap and water and disinfected with 2% Lyorthol after infection*

Time after disinfection (min.)	Isolation of *Pseudomonas aeruginosa*	*Alcaligenes faecalis*	*Staphylococcus aureus*
1	+	+	+
$2\frac{1}{2}$	+	+	+
5	+	+	+
$7\frac{1}{2}$	+	+	+
10	+	+	+
$12\frac{1}{2}$	+	+	+
15	+	+	+
20	+	+	+
30	+	+	+
40	+	−	+
50	+	−	+
60	+	−	+
70	+	−	+
80	+	−	+
90	+	−	+
100	+	−	−
110	+	−	−
120	−	−	−

+ = growth; − = no growth.

DISCUSSION

With the agar cylinder method we were able to determine the degree of contamination in patients' rooms, and the influence of certain cleaning methods and the effect of various disinfectants could be accurately determined. It gives reliable information about the number of bacteria-carrying particles on floors. The number of bacteria in such a particle is usually small—about 4 per particle for *Staphylococcus* according to Lidwell, Noble & Dolphin (1959).

To what degree floors play a part in the starting of contaminations and infections cannot be definitely determined. Certainly infections form a serious problem in many hospitals. To counter them, it is necessary to lower the degree of contamination as far as possible. Because floors are to a certain extent gauges for the whole room, it is useful to test the floors. As floors have to be cleaned regularly, it is possible that the disturbance of the dust carries contamination from the floor. Because averting infections is meaningless unless all means of transport of infection are blocked, floors should be treated. In planning this treatment it must be remembered that damp floors increase the risk of *Pseudomonas* infections, since these organisms can multiply rapidly in a humid environment (Lowbury & Fox, 1953). On the other hand, dry floors often contain dust, which may not only be important as a secondary reservoir in infections with *Staph. aureus* (McDade & Hall, 1964), but in which *Ps. aeruginosa* can also live for a long time (Hurst & Sutter, 1966). When cleaning, both factors should be taken into account, which is not always easy. Some investigators advise the use of disinfectants besides

cleaning with soap and water, as the former cause a marked reduction in the number of bacteria (Ayliffe, Collins & Lowbury, 1966; Foster, 1960). We share their views. In our investigation Halamid and liquor cresoli saponatus gave satisfactory results, but the last of these cannot be used in patients' rooms because of its smell. We therefore prefer Halamid. Because this cannot be used on all floors, those floors should be favoured which are made of material not affected by Halamid, such as polyvynylchloride.

SUMMARY

A cylinder filled with medium is described, with which prints are made of floors or other surfaces. After a print has been made a slice is cut off the medium, put in a Petri dish and incubated. With one filled cylinder about twenty prints can be made. The method can be used for quantitative bacteriological testing of floors, and the effect of treatment with disinfectants can also be checked in a simple way. Preference was given to disinfection with Halamid.

I am obliged to Mr A. Feringa, who helped with the construction of the cylinder and with the examination of the floors, to Mr H. de Vries, who made the cylinder, and to Mrs H. F. v. d. Linden-Bonnema and Miss W. Kuiper for their assistance with the bacteriological investigation of the samples.

REFERENCES

AYLIFFE, G. A. J., COLLINS, B. J. & LOWBURY, E. J. L. (1966). Cleaning and disinfection of hospital floors. *Br. med. J.* ii, 442.

CHAPMAN, G. H. (1946). A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. *J. Bact.* **51**, 409.

CRUICKSHANK, R. (1965). *Medical Microbiology*, 11th ed. Edinburgh and London: E. and S. Livingstone Limited.

FOSTER, W. D. (1960). Environmental staphylococcal contamination: a study by a new method. *Lancet* i, 670.

GENTLES, J. C. (1956). The isolation of dermatophytes from the floors of communal bathing places. *J. clin. Path.* **9**, 374.

HURST, V. & SUTTER, V. L. (1966). Survival of *Pseudomonas aeruginosa* in the hospital environment. *J. infect. Dis.* **116**, 151.

LIDWELL, O. M., NOBLE, W. C. & DOLPHIN, G. W. (1959). The use of radiation to estimate the numbers of micro-organisms in air-borne particles. *J. Hyg., Camb.* **57**, 299.

LOWBURY, E. J. L. & FOX, J. (1953). The influence of atmospheric drying on the survival of wound flora. *J. Hyg., Camb.* **51**, 203.

MCDADE, J. J. & HALL, L. B. (1964). Survival of *Staphylococcus aureus* in the environment. II. Effect of elevated temperature on surface-exposed staphylococci. *Am. J. Hyg.* **80**, 184.

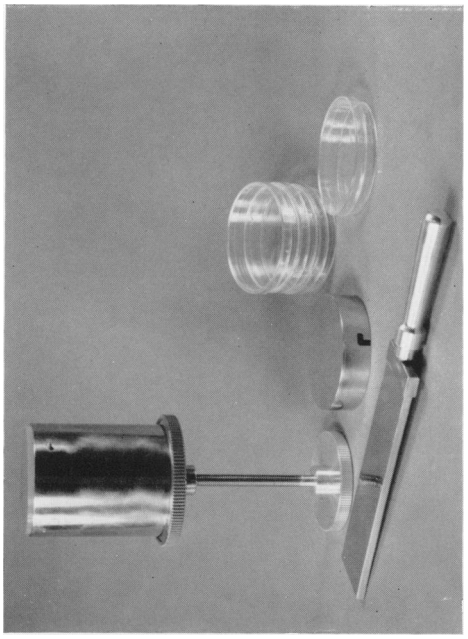
NOBLE, W. C. & LIDWELL, O. M. (1963). Environmental contamination. In *Infections in Hospitals*. Ed. Williams and Shooter. Oxford: Blackwell Science Publications.

RILEY, R. L. & O'GRADY, F. (1961). *Airborne Infection*. New York: The Macmillan Co.

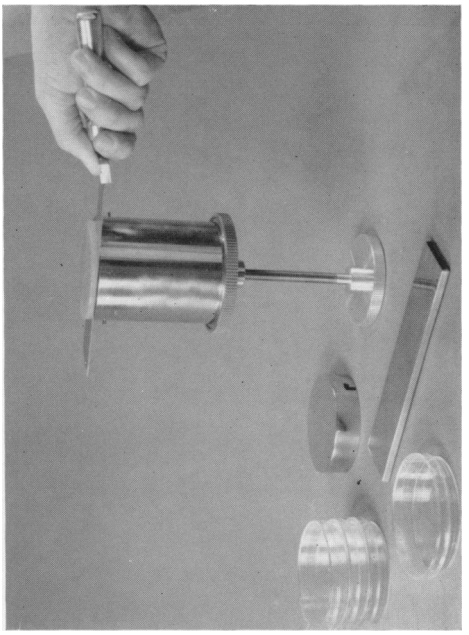
TEN CATE, L. (1965). A note on a simple and rapid method of bacteriological sampling by means of agar sausages. *J. appl. Bact.* **28**, 221.

WILLIAMS, R. E. O. (1949). Examination of floor dust for haemolytic streptococci. *J. Hyg., Camb.* **47**, 416.

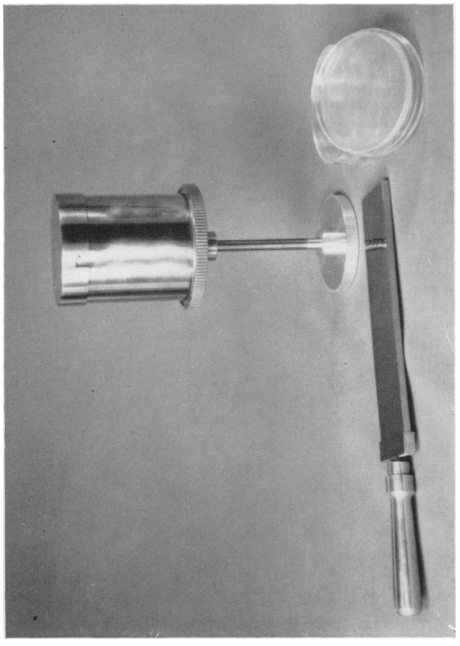
WILLIAMS, R. E. O., BLOWERS, R., GARROD, L. P. & SHOOTER, R. A. (1966). *Hospital Infection*, 2nd ed. London: Lloyd-Luke Ltd.



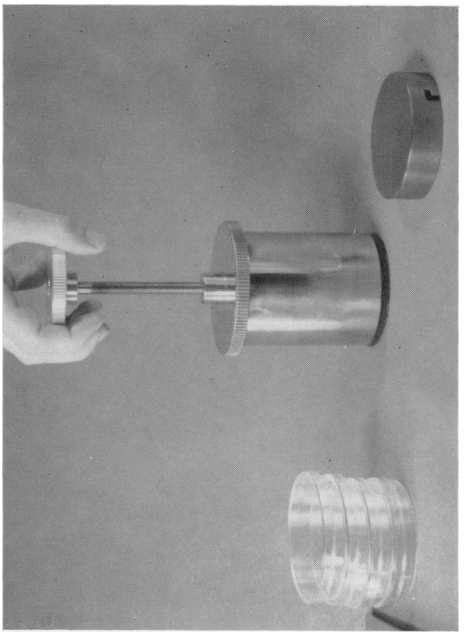
B



D

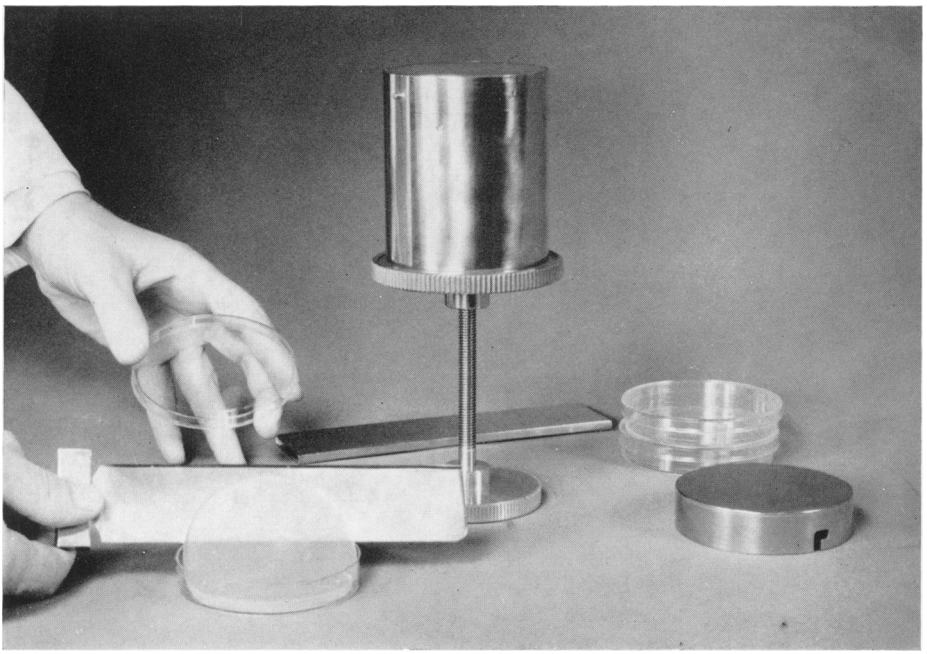


A

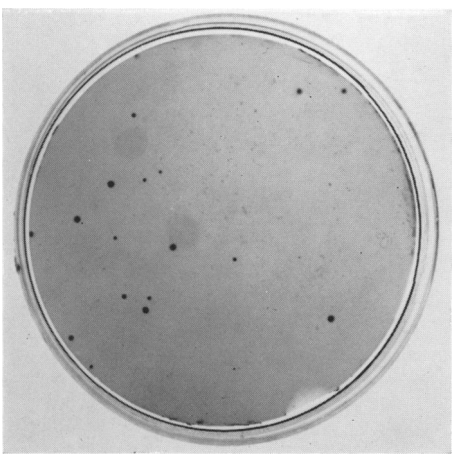


C

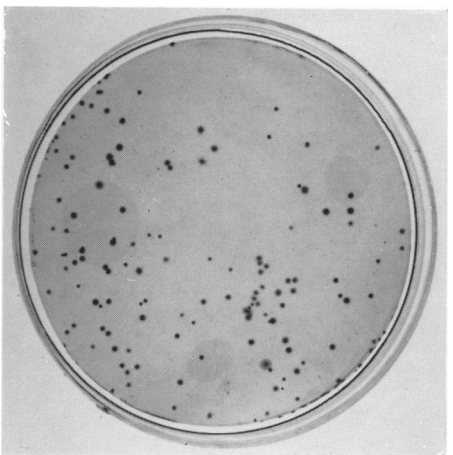
(*Facing p.* 630)



A



B



C

EXPLANATION OF PLATES

Plate 1

A Agar cylinder with accessories.

B Cylinder with medium pushed out 0·5 cm.

C The medium is pressed on the floor to be examined.

D A slice, 0·5 cm. thick, is cut off.

Plate 2

A The cut slice is placed in a Petri dish.

B Print of a floor cleaned with soap and water, immediately after treatment.

C Print of the same floor, 10 hr. after cleaning with soap and water.