

Results and Discussion

Air-flow measurements at the working aperture showed an air speed of 0.137 m/second. After panels had been fitted to reduce the aperture from 84 × 13 cm to the minimum practicable size of 31 × 13 cm, the flow was increased to 0.32 m/second—still less than the recommended rate of 0.5 m/second.

Tests of the furnace efficiency (Table, plates 1-4) showed that viable *Str. hominis* survived in the effluent air and were discharged into the laboratory. Calculations show that to heat the exhaust air from 20°C to the claimed 400°C would need a 4,300 W heater, instead of the 750 W unit provided. This would be equivalent to running at least a four-bar electric fire in the laboratory!

Plate No.	Site and Duration of Exposure	No. of Colonies
1	At exhaust, 3 minutes before test period	1
2	At exhaust, 1 minute during " "	105
3	At exhaust, 3 minutes during " "	293
4	At exhaust, 3 minutes after " "	6
5	Inside cabinet throughout test period	Confluent growth
6	Inside cabinet throughout test and sterilization periods	47

Table Number of colonies of *Str. hominis* on sucrose-agar sampling plates

Survival of the test organisms on plate 6 indicated failure of 30-minute ultraviolet irradiation to sterilize exposed surfaces in the cabinet. Organisms on surfaces shadowed from the lamp showed a proportionately greater survival.

I do not suggest that all furnace-type cabinets would perform as badly as this one, nor do I suggest how this particular model could be improved. These findings are reported to indicate the need for testing equipment of this kind before accepting it as part of the safety arrangements in a laboratory.

I would like to thank Dr R. Blowers, Miss L. Owens, Miss B. Pentland, Mrs M. Wood, and Mr E. Wallington for their help.

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Use of ultraviolet light in the typing of *Pseudomonas aeruginosa* strains by pyocin production

L. LOVREKOVICH, H. LOVREKOVICH, AND D. C. JENKINS
 From the Clinical Laboratories, Department of Pathology, University of Kansas Medical Center, Kansas City, Kansas, USA.

Pseudomonas aeruginosa isolates can be successfully identified by their pyocin production (Darrel and Wahba, 1964; Gillies and Govan, 1966). This warranted the introduction of pyocin typing into our laboratory as a method for studying the epidemiology of *P. aeruginosa* infections. In the course of this study it was found that the method generally used for typing bacteria by pyocin production could be improved. Since we obtained good results with this improved technique it seemed justified to describe the modification in this paper.

Materials and Methods

The typing of the *P. aeruginosa* isolates was carried out by the modified method of Abbott and Shannon (1958) and Gillies (1964) in the following way.

The strain to be typed, the so-called producer, was streaked in a single line on a Mueller-Hinton¹ agar plate in a Petri dish. After incubation for 18 hours, at 37°C, the bulk of the bacteria was removed from the agar with a glass slide. The bacteria which remained on the surface of the medium were first evenly spread with a moist swab, and subsequently killed by exposing the surface of the agar to ultraviolet light. Under our conditions, sterilization was effective after exposing the uncovered plates to irradiation for five minutes at a distance of 40 cm from the germicide lamp. After sterilization six strains, so-called indicators, were tested for their sensitivity to the produced pyocin. These strains were grown for four hours, at 37°C, in a trypticase soy broth before they were streaked at right angles to the first line of inoculation on the agar plate. After incubation for 18 hours, at 37°C, the inhibition of the indicator strains at the site of the accumulated pyocin was observed.

Results

The modification of the typing method consists of

¹Baltimore Biological Laboratory, Cockeysville, Maryland, U.S.A.

Received for publication 29 April 1971.

the use of ultraviolet light instead of chloroform vapour for the sterilization of the pyocin-containing agar plates.

To prove the reliability of the modification parallel tests were performed with the modified and with the original technique.

According to the original method the pyocin-producing bacteria are killed by exposure for 15 minutes to chloroform vapour. To remove the traces of chloroform from the medium after sterilization, the agar plates are exposed to the open air for 10 minutes (Abbott and Shannon, 1958; Gillies, 1964).

The same results were obtained in 100 pyocin sensitivity tests performed with the original and the modified method, showing that the ultraviolet light can be substituted for chloroform.

The ultraviolet light does not alter the activity of the pyocin. This was determined in experiments showing that an exposure of the pyocin to ultraviolet radiation, even for 30 minutes, did not change its effect on the growth of the indicator strains.

It was found that, in contrast to chloroform, the ultraviolet treatment not only kills bacteria but inactivates phages. Therefore, sterilization with ultraviolet light ensures that the inhibition of the indicator bacteria is caused exclusively by the pyocin and not by a phage deriving from the producer strain.

Conclusions

The introduction of ultraviolet light into the pyocin

typing method instead of the application of chloroform has the following advantages. It makes the method faster. Treatment with chloroform requires 25 minutes, but ultraviolet light can do it in five. The use of ultraviolet light results in a cleaner procedure than the manipulation with chloroform. The sterilization by ultraviolet light makes typing not only in glass but in plastic Petri dishes possible. The plastic dishes are attacked by chloroform.

Chloroform does not affect the infectivity of phages. The application of ultraviolet light not only kills the bacteria but also inactivates phages. This eliminates the possibility that the contamination of the producer strain with a phage could lead to infection and growth inhibition of the indicator strains resulting in false reactions on pyocin sensitivity.

It must be pointed out that the even spreading of the producer strain with a moistened swab before exposure to ultraviolet light is a prerequisite for successful sterilization with the radiation. If this is not done the bacterial flora will be too thick for adequate penetration by the ultraviolet light.

References

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