APPLIED MICROBIOLOGY, Mar. 1972, p. 441-443

Vol. 23, No. 3
Printed in U.S.A.

Effect of Flaming Cotton-Plugged Tubes Upon the Contamination of Media During Culture Transfers

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Received for publication 11 August 1971

The effect of flaming the mouth of cotton-plugged broth tubes upon the contamination of media during culture transfers was studied. It was shown that flaming did not reduce contamination significantly either under usual laboratory conditions or when the laboratory atmosphere had been seeded with a test organism so as to achieve higher rates of colony-forming settling particles.

Contradictory indications concerning sterile tube inoculation technique are to be found in a number of textbooks. Some recommend sterilization of the mouth of the tube by flaming (2, 5, 6-8, 10); others would have one hold the tube upside down while introducing the loop (4), while some do away with flaming altogether (1, 3, 9, 11).

Accepted current practice is to flame the mouth of the tube, especially when cotton-plugged, before and after inoculation. The usual exposure of the tube to the flame would actually seem ineffectual in assuring sterilization, especially in the case of bacterial spores. On the other hand, heating the tube expands part of the air within it, and, as it cools while the sterile loop is being introduced, the contracting air might be instrumental in drawing in unwanted microbes. Not having been able to find an experimentally supported reason for the practice of flaming tubes, we decided to bring this to a test.

MATERIALS AND METHODS

Nutrient broth in 5-ml amounts was dispensed into cotton-plugged tubes (15 by 125 mm) and autoclaved at 120 C for 15 min. Two hundred tube lots were divided into an equal number of "donors" and "recipients"; while one operator transferred loopfuls of media from donors to recipients in half of the tubes, employing conventional flaming technique, the other operator handled the other half without flaming.

In the three experiments carried out, a total of four persons alternated in the transfer operations to compensate for individual differences in technique. When flaming was employed, it took 20 to 25 min to complete the transfers, and some 5 min less without flaming. Each 200-tube lot was processed on a different day.

Concurrently with the handling of the tubes, settling plate colony counts were obtained by using at least duplicate sets of nutrient agar plates exposed for different periods of time and incubated at 25 C. The three experiments differed as follows.

Experiment 1. The nutrient broth tubes (1,000 tubes in lots of 200) were tested for sterility by incubating them at 37 C for 24 hr. Thereafter, they were kept at room temperature for an unrecorded length of time until used.

After the transfer operation was completed, the tubes were held at 37 C for 48 hr and then were inspected for appearance of growth.

Experiment 2. The broth tubes (1,200 in lots of 200) were kept on the laboratory bench for no less than 1 week, both to test for sterility and to let them gather dust and contaminants.

After the transfer operation, they were incubated at 25 C for another week and then were inspected for growth.

Experiment 3. The purpose of this experiment was to ascertain the effect of higher rates of settling of airborne microbes upon the contamination rates of broth tubes subjected to the transfer operation with and without flaming. To this effect, we soaked a hand-towel in about 0.5 liter of a 1:5 dilution of a 24-hr nutrient broth culture of *Micrococcus luteus*. The towel was allowed to dry completely in the laboratory atmosphere.

Before the run of each of the six 200-tube lots, the air in the laboratory was freed of settling particles by spraying a mist of water and allowing 10 to 15 min for the drops to settle; ambient humidity was close to saturation at the time of these runs. At this stage, four control plates were exposed for 10 min to verify the effectiveness of particle removal. Then, the towel was shaken vigorously for about 1 min to seed the air with organisms. A small electric fan was set to op-

erate at a 45° vertical angle, thus producing a more uniform distribution of the organisms in the atmosphere.

The transfer operations were started at different times (5 to 30 min) after seeding. In this manner, it was possible to attain different degrees of exposure to an atmosphere in which the number of colony-forming particles settling per minute diminished as time passed. Due to the uniformity of the contaminating population, settling plate counts could be obtained after only 24 hr of incubation at 25 C. After transfer, broth tubes were inspected and tallied after they had undergone a 5-day incubation period at 25 C.

RESULTS AND DISCUSSION

The first experiment attempted to establish whether flaming the tubes at the time of culture transfer would be accompanied by any significant difference in the number of contaminants entering the tubes. The results obtained in this experiment, involving a total of 1,000 broth tubes, may be seen in Table 1; in this, as well as in the other two experiments, the different 200-tube runs were arranged in the table in order of increasing contaminant settling count. Insofar as only one of the tubes showed contamination, it could be concluded that the practice of flaming tubes in culture transfers might be discarded without any practical consequence.

TABLE 1. *Effect of flaming tubes during culture transfers, determined at different levels of air contamination*

Expt	Settling rate[a] (colonies/plate/min)	Per cent broth tubes contaminated when inoculated	
		With flaming	Without flaming
1	0.3	0	0
	0.7	0	0
	0.9	1	0
	1.1	0	0
	1.3	0	0
Average	0.86	0.2	0
2	1.8	0	0
	1.9	2	3
	2.0	0	1
	2.7	2	1
	4.3	2	1
	4.5	3	4
Average	2.9	1.5	1.7
3	20	1	2
	35	7	8
	130	8	9
	200	17	19
	260	14	10
	380	21	24
Average	170	11.3	12.0

[a] At the beginning of each 200-tube run.

The question was raised, however, as to the validity of the results, inasmuch as the 37 C, 48-hr incubation period precluded the manifestation of air contaminants which either could not grow at that temperature or would not form visible growth during such a short incubation period. In addition, it might be desirable to let the tubes pick up dust on the laboratory bench for a week before transfers were made. These questions were answered by the second experiment. Table 1 shows that the new conditions afforded a better opportunity for contaminants to show up; yet, they did so at the rate of 9 contaminated tubes out of 600 handled with flaming as against 10 out of 600 transferred without flaming. The average contaminant settling rate in these runs was more than three times higher than that encountered in the first experiment.

The question remained as to what would happen if the air were laden with contaminants at markedly higher levels. The results of experiment 3 presented in the table show that, with the exception of one 200-tube lot, the total number of contaminated tubes in each run increased in direct relation to the settling plate count at the time; the results also show that this increased number of contaminated tubes is almost equally distributed among the flamed and unflamed lots.

We conclude, therefore, that flaming the mouth of cotton-plugged culture tubes in transfer operations does not reduce contamination significantly and, hence, that this heretofore unquestioned ritual may represent only a waste of time.

The information gained through these experiments, on the other hand, lends support to the practice of holding the culture tubes in a position inclined as close as possible to the horizontal to reduce the effective area of interception of settling particles.

ACKNOWLEDGMENTS

We thank our students Jorge Collado, Carlos Morales, Ronald Arroyo, and José G. Jiménez for their effective assistance.

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