The method to evaluate antibacterial effects of nanoimprinted moth-eye film in practical settings

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Kiyoshi Minoura[1], Miho Yamada[1]

[1]Sharp corporation

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Kiyoshi Minoura

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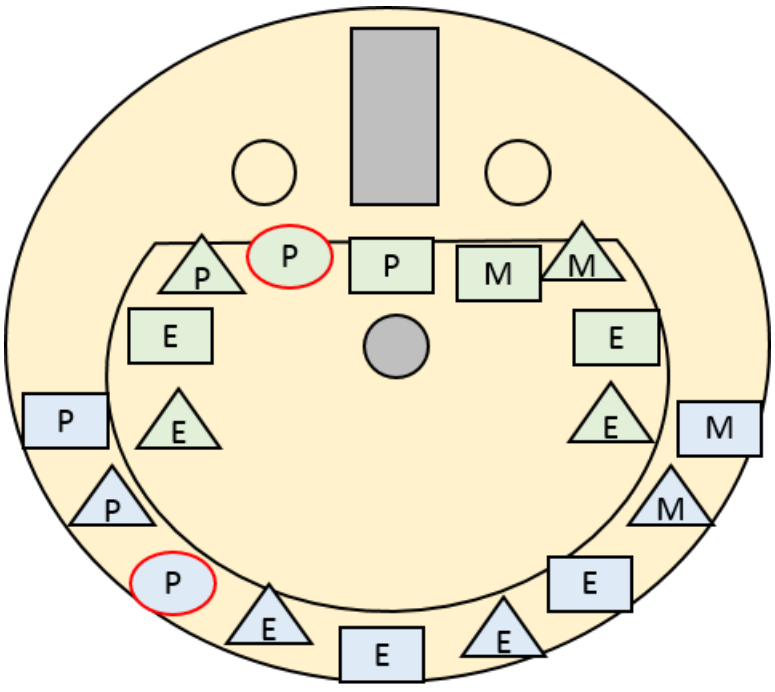
PROTOCOL STATUS

Working

Laminating sample film

1 Sample films, each consisting of a 4-cm-square section of the respective material, were pasted onto the surface of the respective sink on a vertical surface inside of the sink and on a horizontal surface at the fringe of the sink. The arrangement of sample species were changed for each test in order to prevent the number of collecting colonized from being influenced from the pasting place.



2 To adjust the test start time, after laminating, each of the sample film surfaces and each sinks were disinfected by wiping three times with an ethanol-impregnated paper cloth, and that time was taken as the start time of each tests.

Collecting bacteria and counting colonies

3 After testing time, bacteria were collected by culture media. Each sample film was stamped by one stamper, using stampers smaller than the size of the sample film. The contact time of the stamper on the film was approximately three seconds. Three types of stampers, plate count agar (PCA) made of standard agar medium, mannitol salt with egg yolk agar (MSEY), and *Escherichia coli* (ES) Colimark agar (ESCM) were used (Eiken Chemical Co. Ltd., Japan).

4 The culture media stampers were incubated for 48 ±3 hours at 35 degree.

5 The number of colonies that were grown on stampers was counted. To ensure fairness of the examination, the sampler who collected bacteria with stampers and the measurer who counted number of colonies were assigned separately.

The rules of counting

6 For all cases where the colonies were too numerous to count accurately, the number was defined as too-numerous-to-count (TNTC) and set at 400 for averaging purposes. Colonies that covered a relatively large area in the agar were omitted from the counts.

7 Colonies that were visible as dots on PCA medium were identified as common bacteria.

8 Colonies on MSEY medium consisting of white dots and yellow dots were identified as *S.* group and *S. aureus*, respectively. If a colony was difficult to identify as *S. aureus*, the latex agglutination test was performed to decide whether the colony was *S. aureus* or not.

9 Colonies on this medium consisting of blue dots and red dots were identified as *Escherichia coli* or coliform bacteria, respectively.