# The method to evaluate antibacterial effect of moth-eye films

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ABSTRACT

This method used a model designed to represent its practical application.

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## Preparation of microbial suspension

1 Staphylococcus aureus (S. aureus) NBRC12732 strain and Esherichia Coli (E. coli) NBRC3972 strain were kindly supplied by the National Institute of Technology and Evaluation (Tokyo, Japan).

2 S. aureus and E. coli on nutrient agar medium (NA) plate (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) was cultured overnight at 37°C.

3 One colony was selected, inoculated on a new NA plate and cultured again overnight at 37°C

4 The bacteria from the second culture were then suspended in 1/500 nutrient broth (1/500 NB; Eiken Chemical Co. Ltd., Tochigi, Japan) at McFarland Standard 1.0, which contained around 10^8 cfu/ml bacterial cells.

The S. aureus or E. coli suspension was diluted 100 times with 1/500NB to a final bacterial density ranging from

5 2.5×10^6 to 10×10^6 cfu/ml.

## Preparation of sample films

6 The moth-eye stamper was made from aluminum which was anodized and etched. The surface was fabricated into arrays of nano structures whose pitch and height were both approximately 200nm.

7 The resin that composed of urethane trifunctional acrylate having polyethylene glycol (PEG) chains as spacers was spread on base substrate film with 75 µm poly ethylene terephthalate thickness.

8 The substrate film with the resin was laminated with a nip roll on the moth-eye stamper.

9 The resin was exposed to ultraviolet light, and was peeled off from the moth-eye stamper by hand after the completion of curing. The peeled film was used as sample A.

10 As for flat surface films (Sample B), the moth-eye stamper was substituted to the flat glass stamper. The film was fabricated with the same manner with step 9. The peeled film was used as sample B.

11 PET film was used as a control film.

12 All sample films were cut out in 5cm square.

13 All sample films were disinfected with ethanol.

## Antibacterial assay of droplet model

14 The sample film was placed in the center of the petri dish.

15 Ten µl of the final microbial solution was dropped onto a sample film and kept in a safety cabinet at room temperature for the indicated time.



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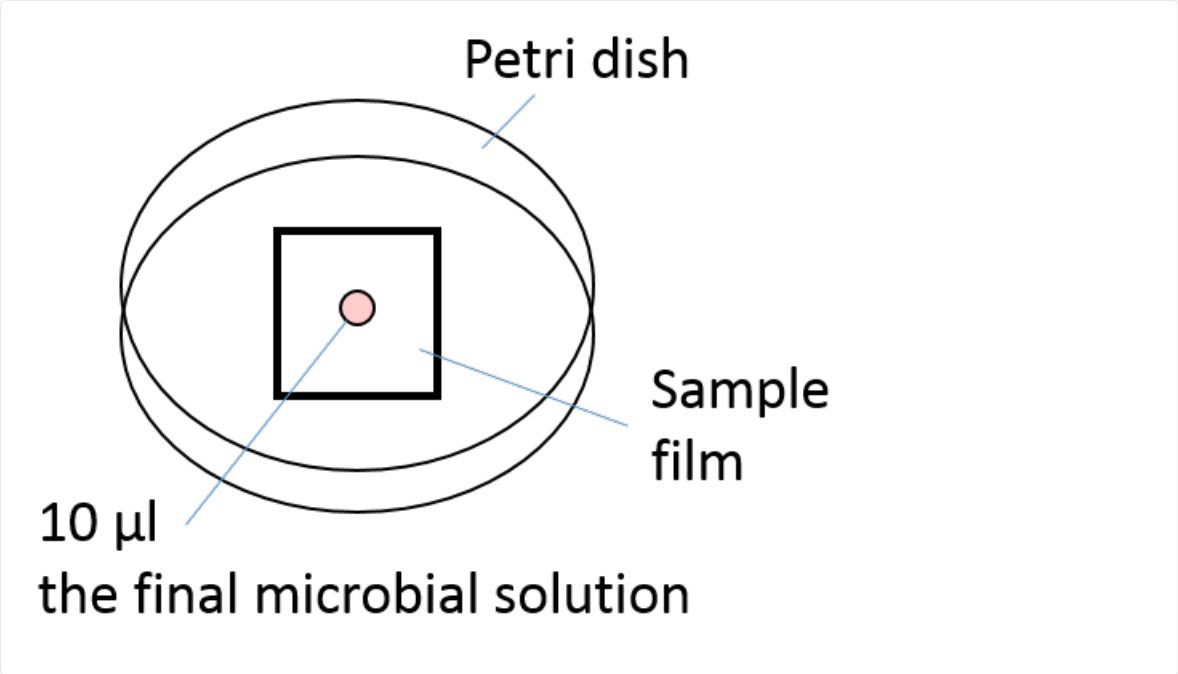
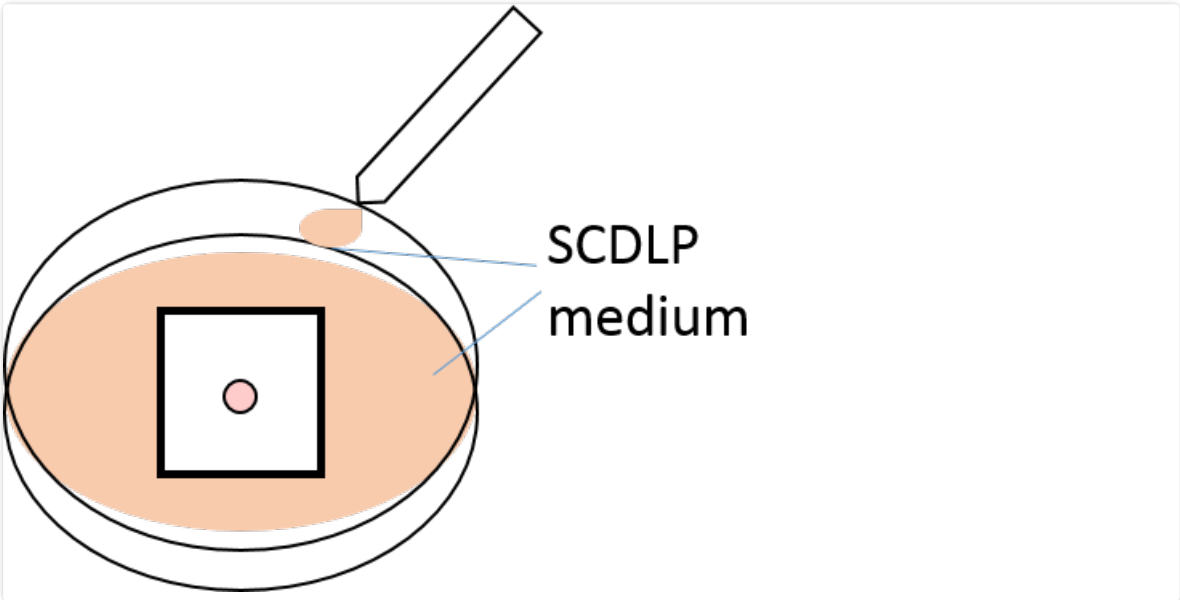



16 The medium consisting of 9.6 ml of fresh soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP; Wako Pure Chemical Industries Ltd., Osaka, Japan) was poured directly onto the sample film in the petri dish, and the bacteria attached to the film surface were washed out by shaking the petri dish for 30 sec at 500 rpm/min using a vortex mixer.




17 The sample film was washed out again with the SCDLP medium collected form the petri dish. This process was repeated three time.

18 All the SCDLP medium in petri dish, which was around 10ml, was collected in the 50mL conical centrifuge tube.



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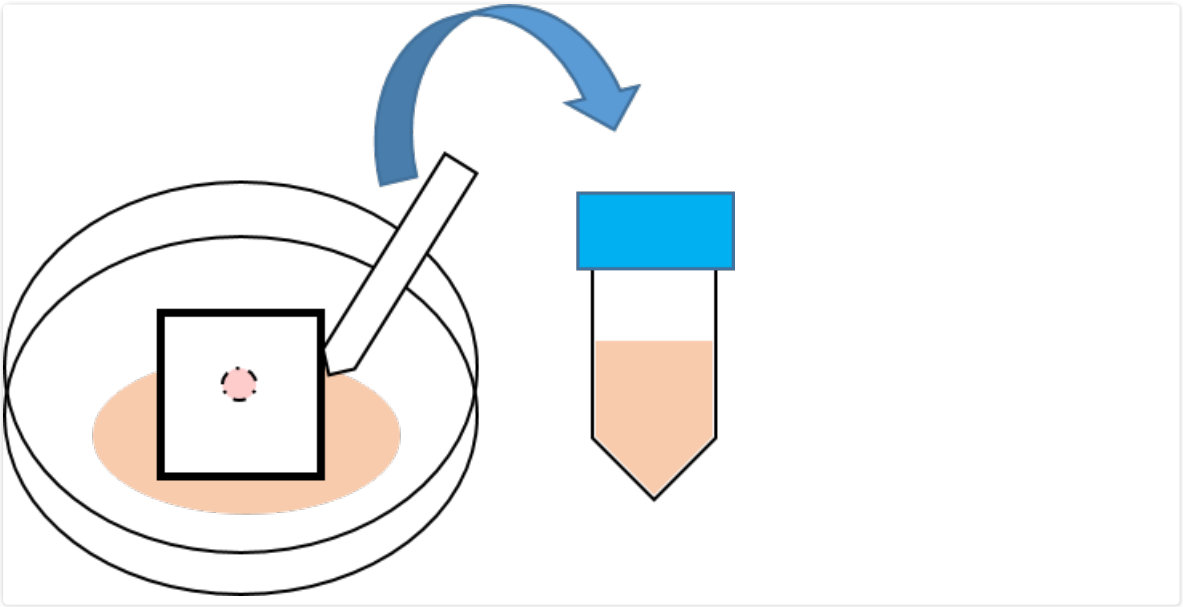

19 The bacterial suspension was then serially 10-fold diluted with PBS. One ml of each diluted sample was quickly mixed with 9 ml of melted Pearl Core plate count agar (PC; Eiken Chemical Co. Ltd., Tokyo, Japan) kept at 50°C in a water bath, and poured onto a petri dish and cooled down to room temperature.

20 The bacteria suspension in Pearl Core plate count agar was cultered 24 hours at 37°C and the number of visible colony in the dish was counted.



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