ISO 22196
Measurement of antibacterial activity on plastics and non-porous surfaces

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Japan Food Research Laboratories

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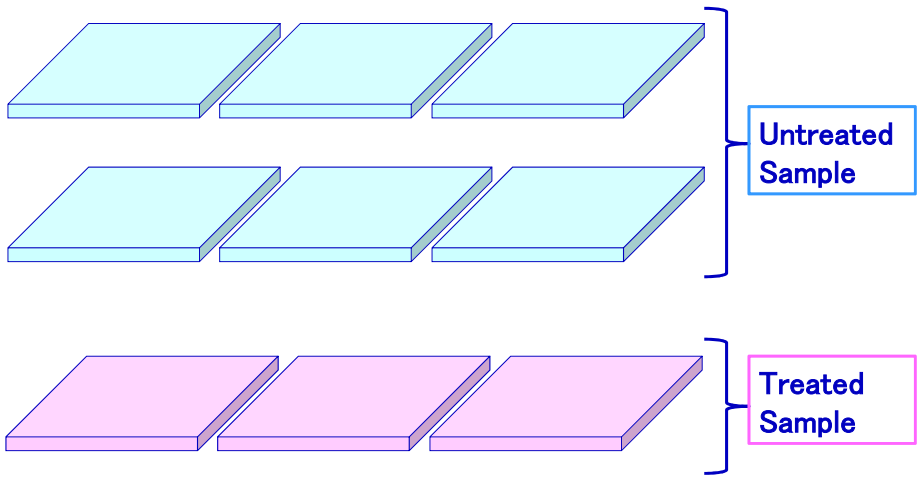
Test protocol of ISO 22196



2

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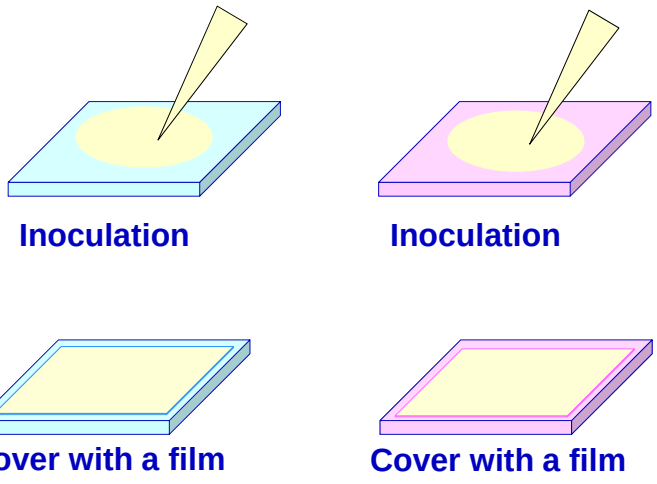
Test protocol of ISO 22196



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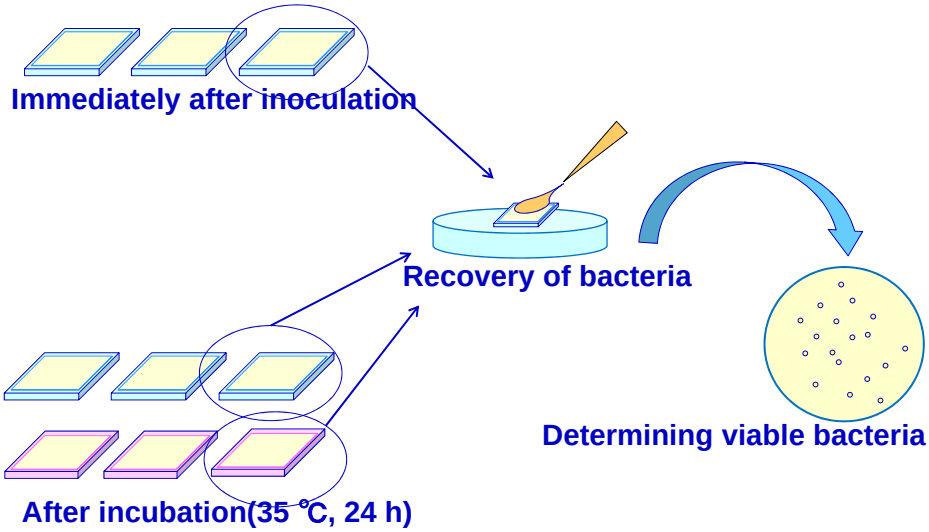
Test protocol of ISO 22196



4

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4.1 Bacteria to be used for the tests

Both of the following species of bacteria shall be used.

a) *Staphylococcus aureus*
ATCC 6538P, CIP 53.156, DSM 346, NBRC 12732, NCIB 8625

b) *Escherichia coli*
ATCC 8739, CIP 53.126, DSM 1576, NBRC 3972, NCIB 8545

Both strains are Biosafety Level 2 (BSL 2).

5

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4.2 Reagents, culture media and solutions

Water shall be distilled or deionized and have a conductivity of < 1 µS/cm.

All reagents shall be of analytical grade and/or of a grade appropriate for microbiological purpose.

Note
The deionizer and reverse-osmosis units are used to produce distilled or deionized/demineralized water of the required quality.

6

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4.2.3 Culture

4.2.3.1 General

The medium may be obtained from commercial suppliers, in which case it shall be prepared for use in accordance with the manufacturer's instructions.

7

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4.2.3 Culture

Recommendation

The culture media after opening should be stored in the desiccator which kept low humidity.

8

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4.2.3 Culture

4.2.3.2 Suspension medium - 1/500 nutrient broth (1/500 NB)

Dilute the nutrient broth with distilled or deionized water to a 500-fold volume and adjust the pH to a value between 6,8 and 7,2 with sodium hydroxide or hydrochloric acid.

Sterilize by autoclaving.

If it is not used immediately after preparation, store it at 5 ℃ to10 ℃.

A 1/500 NB that has been kept for one week or longer after preparation shall not be used.

9

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5. Apparatus

5.7 Incubator

Recording thermocouple

Lock

Record

10

Note

Refrain from leaving the incubator door open for long time.

Check the temperature at least every working day.

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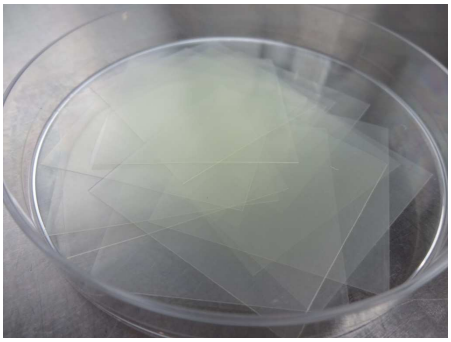
5. Apparatus

5.11 Cover film



11

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6. Sterilization of apparatus and storage of stock cultures

6.4 Maintenance of stock cultures

Stock cultures shall be stored at 5 ℃ to 10 ℃ on an appropriate medium and transferred monthly. After five transfers or if more than one month has passed between transfers, the stock culture shall be discarded and replaced with a fresh culture obtained from the institute or culture collection concerned.

12

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6. Sterilization of apparatus and storage of stock cultures




Deep-frozen storage cultures

Deep freezer (-80 ℃)

13

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6. Sterilization of apparatus and storage of stock cultures


Stock cultures (5 ℃ to 10 ℃)

Refrigerator

14

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7. Procedure

7.1 Pre-culture of bacteria

Transfer bacteria from the stock culture to the slant culture medium and incubate at (35±1) ℃ for 16 h to 24 h.

From this culture, transfer bacteria onto fresh slant culture medium and incubate (35±1) ℃ for 16 h to 20 h.

15

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7. Procedure

7.2 Preparation of test specimens

Cleaning of the test specimen can cause softening, dissolution of the surface coating or elution of components, so should be avoided. If cleaning is required due to gross contamination, the cleaning method shall be stated in the test report.

16

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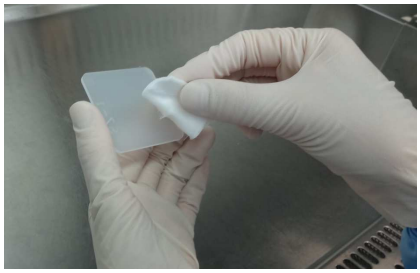
7. Procedure

7.2 Preparation of test specimens
Cleaning



17

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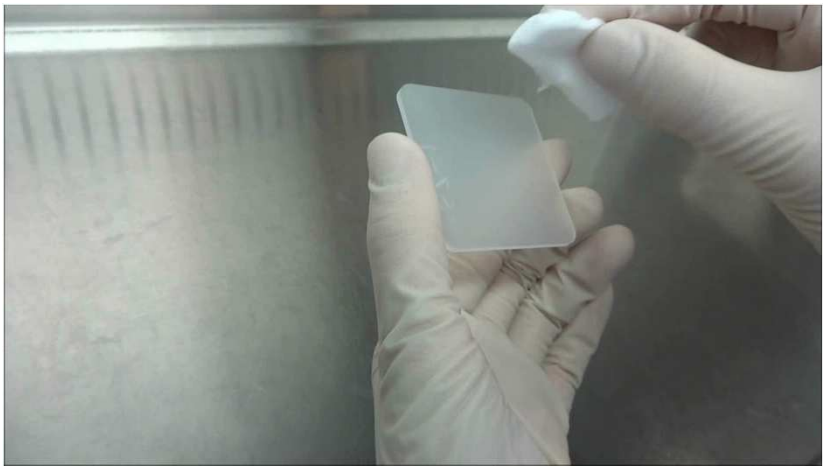
7. Procedure

7.2 Preparation of test specimens
Cleaning



18

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7. Procedure

7.3 Preparation of test inoculum

Transfer one loop of the test bacteria into a small amount of 1/500 NB.

Estimate the number of bacteria using direct microscopic observation and a counting chamber or another appropriate method (e.g. spectrophotometrically).

Dilute this suspension with 1/500 NB to obtain a bacterial concentration that is between 2,5 x 10^5 cells/ml and 10 x 10^5 cells/ml, with target concentration of 6 x 10^5 cells/ml.

19

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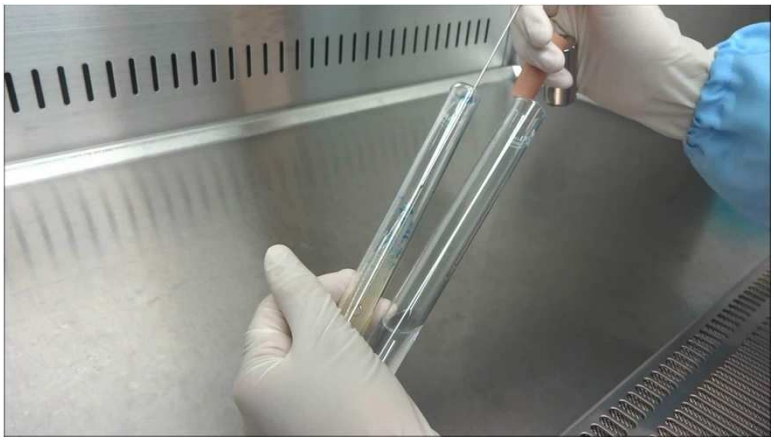
7. Procedure

7.3 Preparation of test inoculum



20

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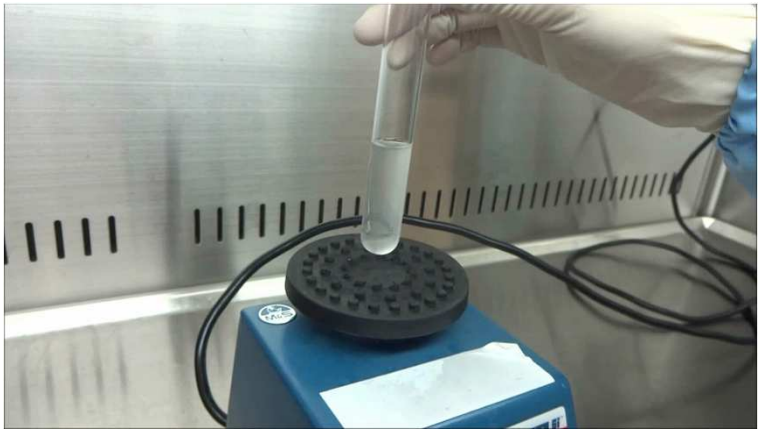
7. Procedure

7.3 Preparation of test inoculum



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7. Procedure

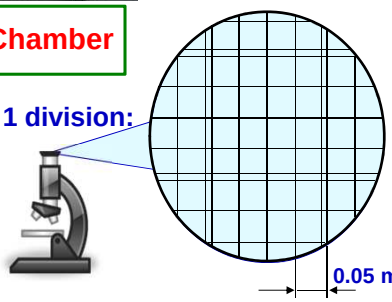
7.3 Preparation of test inoculum

Counting Chamber

Cover glass

The view by the microscope

The volume of 1 division:
5×10^{-8} cm^3



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7. Procedure

7.4 Inoculation of test specimens

Pipette 0,4 ml of the test inoculum onto the test surface.

Cover the test inoculum with a piece of film(40 mm x 40 mm) and gently press down on the film so that the test inoculum spreads to, but dose not leak beyond, the edges of the film.

23

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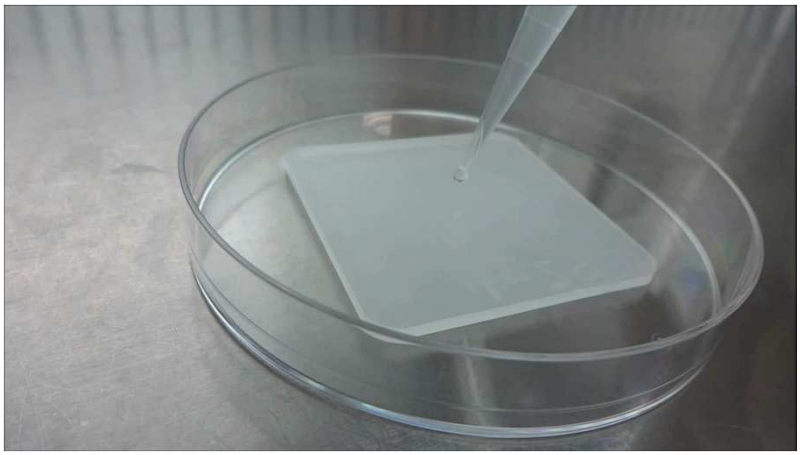
7. Procedure

7.4 Inoculation of test specimens



24

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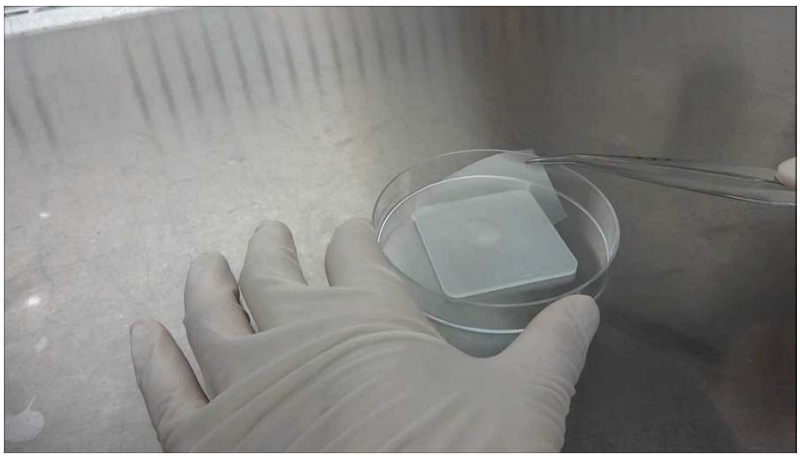
7. Procedure

7.4 Inoculation of test specimens



25

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7. Procedure

7.5 Incubation of the inoculated test specimens

Incubate the Petri dishes containing the inoculated test specimens at a temperature of (35 ± 1) ℃ and a relative humidity of not less than 90 % for (24 ± 1)h.

26

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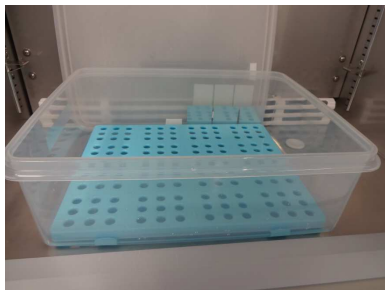
7. Procedure

7.5 Incubation of the inoculated test specimens



27

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7. Procedure

7.5 Incubation of the inoculated test specimens

28

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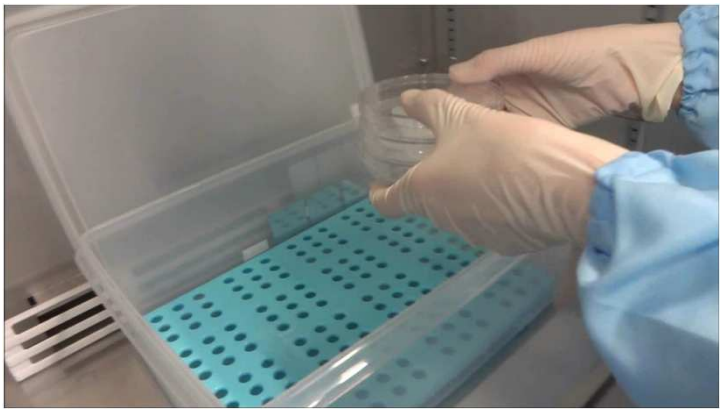

29

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7. Procedure

7.6 Recovery of bacteria from test specimens

7.6.1 Test specimens immediately after inoculation

Immediately after inoculation, the untreated test specimens by adding 10 ml of either SCDLP broth or suitable, validated neutralizer to petri dish containing the test specimen.

It is important to ensure that the neutralizer completely washes the specimens by using a pipette to collect and release the SCDLP broth at least four times.

30

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7. Procedure

7.6.2 Test specimens after incubation

After the incubation, process the remaining test specimens by adding 10 ml of either SCDLP broth or suitable, validated neutralizer to petri dish containing the test specimen.

It is important to ensure that the neutralizer completely washes the specimens by using a pipette to collect and release the SCDLP broth at least four times.

31

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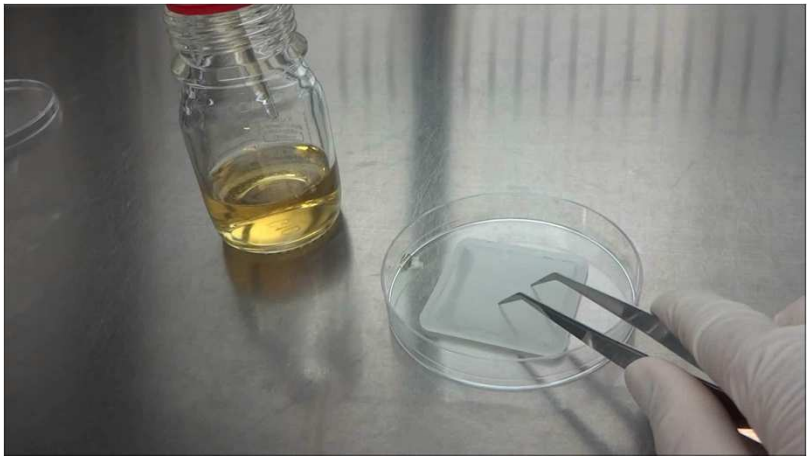
7. Procedure

7.6 Recovery of bacteria from test specimens



32

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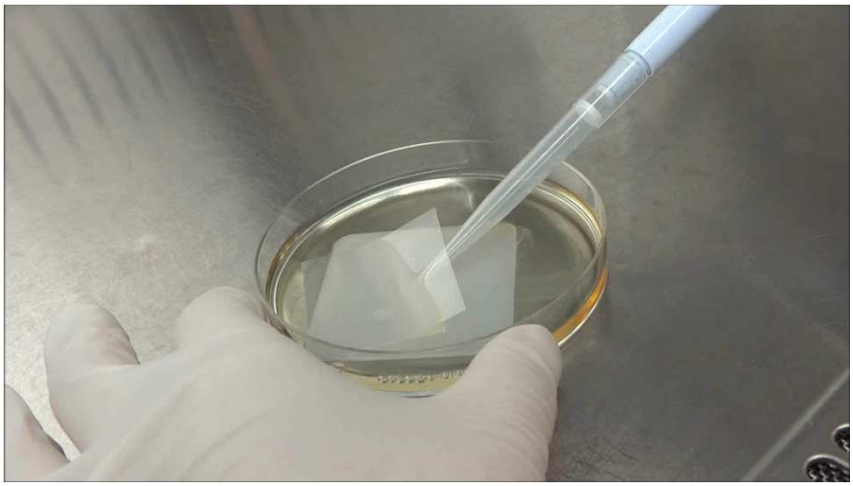
7. Procedure

7.6 Recovery of bacteria from test specimens



33

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7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method

Enumerate viable bacteria by performing 10-fold serial dilutions of the SCDLP in phosphate-buffered physiological saline. Place 1 ml of each dilution, as well as 1 ml of SCDLP recovered from the test specimens, into separate sterile Petri dish. Pour 15 ml of plate count agar into each Petri dish and swirl gently to disperse the bacteria. All plating shall be performed in duplicate. Replace the lids, invert the Petri dish and incubate them at (35 ± 1) ℃ for 40 h to 48 h.

34

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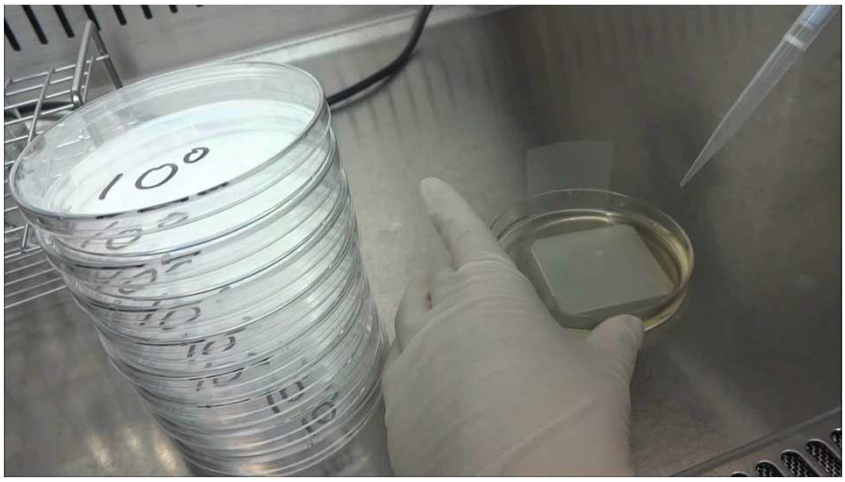
7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method



35

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7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method

36

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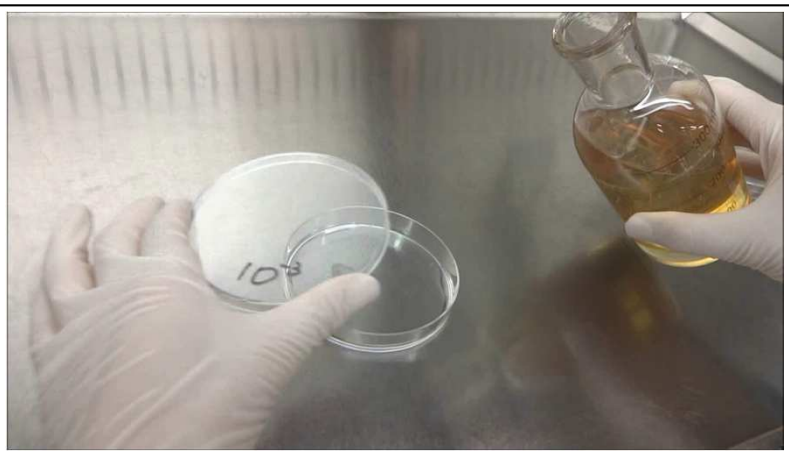

37

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7. Procedure

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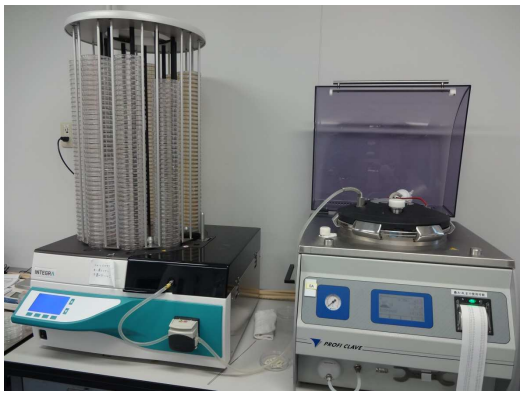

Media preparatpr

38

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7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method

39

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7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method

After incubation, count the number of colonies in the Petri dishes containing 30 to 300 colonies. For each dilution series, record the number of colonies recovered to two significant figures, as well as the dilution factor for the plates used for counting. If the number of colonies in the plates containing the 1 ml aliquots of SCDLP is less than 30, then count and record the number of colonies in these plates. If there are no colonies recovered in any of the agar plates in the dilution series, record the number of colonies as “< 1”.

40

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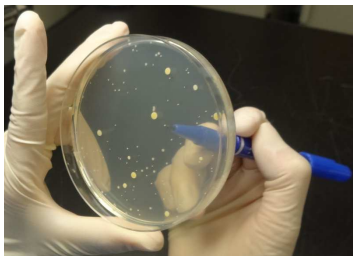
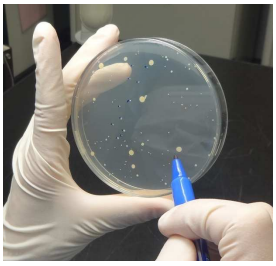
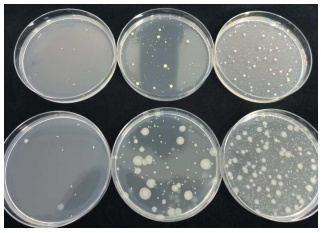
7. Procedure

7.7 Determining the viable bacteria count by the pour plate culture method







41

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7. Procedure

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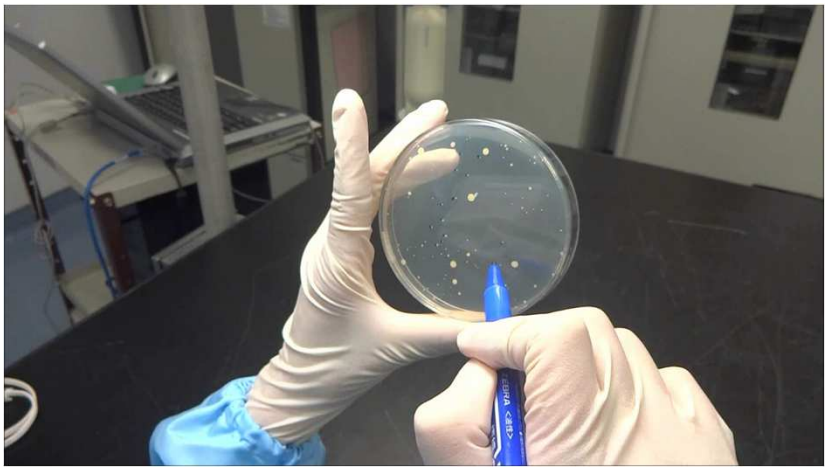

42

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8. Expression of rsults

8.1 Determination of the number of viable bacteria

For each test specimen, determine the number of viable bacteria recovered in accordance with Equation(1):

$$N = (100 \times C \times D \times V)/A \quad (1)$$

N: The number of viable recovered per cm^2 per test specimen
C: The average plate count for the duplicate plates
D: The dilution factor for the plates counted
V: The volume(ml) of SCDLP added to the specimen
A: The surface area(mm^2) of the cover film

43

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8. Expression of results

8.2 Conditions for a valid test

When the three conditions respectively are satisfied, the test is deemed valid.

8.2.2 The logarithmic value of the number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall satisfy the following requirement:

$$(L_{max} - L_{min}) / (L_{mean}) \leq 0,2$$

L_{max}: Log_{10} of maximum number of viable bacteria
L_{min} : Log_{10} of minimum number of viable bacteria
L_{mean} : Log_{10} of mean number of viable bacteria

44

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8. Expression of results

8.2 Conditions for a valid test

When the three conditions respectively are satisfied, the test is deemed valid.

8.2.3 The average number of viable bacteria recovered immediately after inoculation from the untreated test specimens shall be within the range 6,2 x 10^3 cells/cm^2 to 2,5 x 10^4 cells/cm^2.

8.2.4 The number of viable bacteria recovered from each untreated test specimen after incubation for 24 h shall not be less than 6,2 x 10^1 cells/cm^2.

45

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8. Expression of results

8.3 Calculation of the antibacterial activity

When the test is deemed valid, calculate the antibacterial activity using the Equation(2), recording the result to one decimal place.

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (2)$$

R: Antibacterial activity
U_0: Ave. of the $\log_{10}$ of the number of viable bacteria recovered from untreated test specimens immediately after inoculation
U_t: Ave. of the $\log_{10}$ of the number of viable bacteria recovered from untreated test specimens after 24 h
A_t: Ave. of the $\log_{10}$ of the number of viable bacteria recovered from treated test specimens after 24 h

46

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8. Expression of results

8.4 Effectiveness of the antibacterial agent

The value of the antibacterial activity can be used to characterize the effectiveness of antibacterial agent. The antibacterial - activity values used to define the effectiveness shall be upon by all interested parties.

47