



JAPANESE
INDUSTRIAL
STANDARD

Translated and Published by
Japanese Standards Association

JIS L 1902 : 2015

(JTETC/JSA)

**Textiles—Determination of
antibacterial activity and efficacy of
textile products**

ICS 07.100.99;59.080.01

Reference number : JIS L 1902 : 2015 (E)

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L 1902 : 2015

Date of Establishment: 1990-11-01

Date of Revision: 2015-07-21

Date of Public Notice in Official Gazette: 2015-07-21

Investigated by: Japanese Industrial Standards Committee

Standards Board for ISO area

Technical Committee on Consumer Life Products

JIS L 1902:2015, First English edition published in 2016-01

Translated and published by: Japanese Standards Association
Mita MT Building, 3-13-12, Mita, Minato-ku, Tokyo, 108-0073 JAPAN

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Printed in Japan

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Foreword

This translation has been made based on the original Japanese Industrial Standard revised by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee according to the proposal for revision of Japanese Industrial Standard submitted by Japan Textile Evaluation Technology Council (JTETC)/Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law. Consequently **JIS L 1902:2008** has been withdrawn and partially replaced with this Standard.

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Textiles—Determination of antibacterial activity and efficacy of textile products

Introduction

This Japanese Industrial Standard has been prepared based on the second edition of **ISO 20743** published in 2013 with some modifications of the technical contents to correspond to technical trend and actual situation in Japan.

The portions given sidelines or dotted underlines are the matters in which the contents of the corresponding International Standard have been modified. A list of modifications with the explanations is given in Annex JB. Testing examples are given in Annex E.

The matters contained in Annex JA are unique contents of JIS that are not given in the corresponding International Standard.

1 Scope

This Standard specifies quantitative and qualitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens and antibacterial efficacy.

This Standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, bedclothes, home furnishings and miscellaneous goods, regardless of the type of antibacterial agent used (organic, inorganic, natural or man-made) or the method of application (built-in, after-treatment or grafting).

Based on the intended application and on the environment in which the textile product is to be used and also on the surface properties of the textile properties, the user can select the most suitable of the following four determination methods on determination of antibacterial activity.

- a) **Absorption method** An evaluation method in which test bacterial suspension is inoculated directly onto the specimens.
- b) **Transfer method** An evaluation method in which test bacteria are placed on an agar plate and transferred onto the specimens.
- c) **Printing method** An evaluation method in which test bacteria are placed on a filter and printed onto specimens.
- d) **Halo method** A qualitative method to evaluate by the existence of halo.

The colony plate count method and the ATP (ATP = Adenosine Tri-phosphate) luminescence method are also specified for measuring the enumeration of bacteria.

The evaluation of photocatalytic antibacterial efficacy treated on the photocatalytic finished textile product shall be carried out according to **JIS R 1702**.

NOTE : The International Standard corresponding to this Standard and the symbol of degree of correspondence are as follows.

ISO 20743:2013 *Textiles—Determination of antibacterial activity of textile products* (MOD)

In addition, symbols which denote the degree of correspondence in the contents between the relevant International Standards and **JIS** are IDT (identical), MOD (modified), and NEQ (not equivalent) according to **ISO/IEC Guide 21-1**.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 0970 *Piston pipettes*

JIS K 3800 *Class II biological safety cabinets*

JIS K 8150 *Sodium chloride (Reagent)*

JIS L 0803 *Standard adjacent fabrics for staining of colour fastness test*

JIS R 1702 *Fine ceramics (advanced ceramics, advanced technical ceramics)—Test method for antibacterial activity of photocatalytic products and efficacy*

JIS R 3505 *Volumetric glassware*

JIS Z 8401 *Guide to the rounding of numbers*

3 Terms and definitions

For the purpose of this Standard, the following term and definition apply.

3.1 control specimen

specimen which is the same textile product as the textile product to be tested, but without antibacterial treatment, used to validate the condition of test effectiveness

If control specimen is not available, a 100 % cotton standard adjacent fabric (cotton No. 3-1) specified in **JIS L 0803** is used as the control specimen, after 10 cycles of washing for 10 min at a temperature of 60 °C without detergents or any brighteners, rinsing twice for 5 min and air-drying.

3.2 antibacterial agent

product designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.3 antibacterial finish

treatment designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.4 antibacterial activity

activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.5 antibacterial efficacy

efficacy of antibacterial activity shown by antibacterial finish

It is evaluated by the existence of halo and antibacterial activity value.

3.6 plate count method

method in which the number of bacteria present after incubation is calculated by counting the number of colonies according to a ten-time dilution method

The results are expressed in “CFU (Colony Forming Unit)”.

3.7 luminescence method

method in which the amount of adenosine triphosphate (hereafter referred to as “ATP”) contained in bacterial cells is measured

The results are expressed in “moles of ATP”.

3.8 neutralizer

chemical agents used to inactivate, neutralize or quench the antibacterial properties of antibacterial agents

3.9 halo

the part where the growth of test bacteria produced around the sample is suppressed when the antibacterial processed sample is placed and incubated on the culture medium containing the test bacteria

4 Safety precaution

The test methods specified herein require the use of bacteria. These tests should be carried out by person with training and experience in the use of microbiological techniques. Safety precautions shall be checked against and comply with regulations.

5 Apparatus

5.1 Spectrophotometer, capable of measuring at a 620 nm to 660 nm wavelength, or McFarland's nephelometer.

5.2 Incubator, capable of maintaining a constant temperature of $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

5.3 Water baths, one capable of maintaining a constant temperature of $46\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ and another capable of maintaining a temperature of $70\text{ }^{\circ}\text{C}$ to $90\text{ }^{\circ}\text{C}$.

5.4 Mixer, producing a vortex shaking action.

5.5 Stomacher, capable of speeds of 6 blows per second to 8 blows per second, with the corresponding disposable containers.

5.6 Clean bench or safety cabinet, for microbial test. Safety cabinet is specified in JIS K 3800.

5.7 Platinum colony loop, with a loop of approximately 4 mm in diameter.

5.8 Humidity chamber, tropical chamber or other container capable of maintaining a high-humidity more than 70 %RH atmospheric condition.

5.9 Luminescence photometer, capable of measuring ATP of 10^{-13} mol/L to 10^{-7} mol/L at 300 nm to 650 nm with a luminescence-measuring reagent.

5.10 Printing apparatus, capable of applying a 4 N load to a test specimen and rotating the specimen 180° in one direction for a period of 3.0 s.

5.11 Refrigerator, capable of maintaining a temperature of between 2 °C and 8 °C.

5.12 Freezers, one adjustable to a temperature below -70 °C and another to a temperature below -20 °C.

5.13 Balance, which can be read to the nearest 0.01 g.

5.14 Filtering apparatus, consisting of an upper container equipped with a membrane filter and a lower container equipped with a suction opening.

5.15 Pipette, having the most suitable volume for each use, with a tip made of glass or plastic, and with a tolerance to conform to Class A in **JIS K 0970** or **JIS R 3505** or equivalent to it.

5.16 Vials, 30 ml glass bottles, with screw openings, polytetrafluoroethylene or silicone packing and caps made of polypropylene, polycarbonate or another suitable material.

5.17 Petri dishes, that have been sterilized, made of glass or plastic, in diameter sizes of 90 mm to 100 mm. For the specimen of transfer method, in diameter sizes of 55 mm to 60 mm.

5.18 Glass rod, with a diameter of approximately 18 mm.

5.19 Anti-bumping granules (glass beads), with a diameter of 3 mm to 4 mm.

5.20 Erlenmeyer flask with cap, of capacity 100 ml.

5.21 Cutting template, made of a sterilizable material (stainless steel or glass) with a diameter of $38 \text{ mm} \pm 1 \text{ mm}$.

5.22 Disposable plastic bags, sterile bags suitable for containing food products, to be used for one of the shaking methods of the specimens.

5.23 Tweezers, made of a material which can be sterilized.

5.24 Stainless-steel cylinder, with a mass of $200 \text{ g} \pm 10 \text{ g}$ and a diameter of $35 \text{ mm} \pm 1 \text{ mm}$.

5.25 Metal wire basket, for autoclaving, to be used for sterilizing test specimens.

5.26 Aluminium foil, capable of wrapping a metal wire basket, etc.

5.27 Reciprocal incubation shaker, capable of adjusting a temperature of $37 \text{ °C} \pm 2 \text{ °C}$, 110 time/min and about 30 mm in amplitude.

5.28 Autoclave, capable of sterilizing at $120 \text{ °C} \pm 2 \text{ °C}$ and $103 \text{ kPa} \pm 5 \text{ kPa}$.

6 Reagents and culture media

6.1 Water Water used in tests shall be analytical-grade water for microbiological media preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with RO (reverse osmosis). It shall be free from all toxic or bacteria inhibitory substances.

6.2 Tryptone soya broth (TSB) Mix well the following compositions and adjust pH to 7.2 ± 0.2 , then sterilize by autoclave (5.28).

— Tryptone, pancreatic digest of casein	17 g
— Soya peptone, papain digest of soya	3 g
— Sodium chloride (NaCl) <u>According to JIS K 8150.</u>	5 g
— Glucose	2.5 g
— Dipotassium hydrogen phosphate	2.5 g
— Water (6.1)	1 000 ml

6.3 Tryptone soya agar (TSA) Mix well the following compositions and adjust pH to 7.2 ± 0.2 , then sterilize by autoclave.

— Tryptone, pancreatic digest of casein	15 g
— Soya peptone, papain digest of soya	5 g
— Sodium chloride (NaCl)	5 g
— Agar	15 g
— Water	1 000 ml

6.4 Agar for transfer Mix well the following compositions and adjust pH to 7.2 ± 0.2 , then sterilize by autoclave.

— Tryptone, pancreatic digest of casein	0.75 g
— Soya peptone, papain digest of soya	0.25 g
— Sodium chloride (NaCl)	5 g
— Agar	15 g
— Water	1 000 ml

6.5 Nutrient broth (NB) Mix well the following compositions and adjust pH to 6.9 ± 0.2 , then sterilize by autoclave.

— Beef extract	3 g
— Peptone	5 g
— Water	1 000 ml

6.6 Peptone salt solution Mix well the following compositions and adjust pH to 6.9 ± 0.2 , then sterilize by autoclave.

— Peptone, pancreatic digest of casein	1 g
— Sodium chloride (NaCl)	8.5 g
— Water	1 000 ml

6.7 Physiological saline Mix well the following compositions and adjust pH to 6.9 ± 0.2 , then sterilize by autoclave.

— Sodium chloride (NaCl)	8.5 g
— Water	1 000 ml

6.8 SCDLP medium Mix well the following compositions and adjust pH to 7.2 ± 0.2 , then sterilize by autoclave.

— Peptone, digest of casein	17 g
— Peptone, digest of soybean	3 g
— Sodium chloride (NaCl)	5 g
— Dipotassium hydrogen phosphate	2.5 g
— Glucose	2.5 g
— Lecithin	1 g
— Polysorbate 80	7 g
— Water	1 000 ml

6.9 Dilution buffer for shake-out bacterial suspension This buffer solution consists of 0.005 mol/L sodium dihydrogen phosphate containing 0.037 % sucrose and adjusting pH to 7.2 ± 0.2 .

6.10 Neutralizing solution The composition of the standard neutralizing solution shall be as follows.

— Polysorbate 80	30 g
— Egg-yolk lecithin	3 g
— Histidine hydrochloride	1 g
— Meat or casein peptone	1 g
— Sodium chloride (NaCl)	4.3 g
— Monopotassium phosphate	3.6 g
— Disodium phosphate dihydrate	7.2 g
— Water	1 000 ml

6.11 Enumeration agar (EA) Mix well the following compositions and adjust pH to 7.2 ± 0.2 , then sterilize by autoclave.

— Dehydrated yeast extract	2.5 g
— Casein tryptone	5.0 g
— Glucose	1.0 g

- Agar 12 g to 15 g¹⁾
- Water 1 000 ml

Note ¹⁾ Necessary mass is depending on the gel strength of the product.

6.12 Agar for printing Mix well the following compositions, then sterilize by autoclave.

- Agar 20 g
- Water 1 000 ml

6.13 Cryoprotective solution for bacterial species For freezing, a cryoprotective solution containing 150 g/L of glycerol or 100 g/L of dimethylsulfoxide shall be used and prepared as follows.

- a) Add 1 000 ml of tryptone soya broth (TSB) (6.2) or nutrient broth (NB) (6.5). Add 150 g of glycerol or 100 g of dimethylsulfoxide, and mix well and sterilize by autoclave.
- b) For solutions containing glycerol, sterilize the mixed solution by autoclave. For solutions containing dimethylsulfoxide, sterilize the mixed solution by using 0.22 µm membrane filter.

NOTE : Any commercially available product may be used as long as it is a cryoprotective solution or preserving system that contains glycerol or dimethylsulfoxide and allows preservation of the strains in the same manner as the specified solutions.

6.14 Stock solution of ATP standard reagent Dissolve 60.5 mg of adenosine-disodium 5'-triphosphate trihydrate with water to make 1 000 ml. The concentration of ATP standard reagent is 1×10^{-4} mol/L which is obtained by the following mixing.

After preparation, the solution shall be placed in a tightly sealed container and cryopreserved at a temperature of -20 °C or lower. The solution shall be used no later than six months from the date of preparation.

Do not refreeze frozen solution that has thawed.

NOTE : The suitable amount of adenosine-disodium 5'-triphosphate trihydrate may be calculated from the ATP content of each commercial product.

6.15 Buffer solution for ATP luminescent reagent, prepared from the following compositions to be pH 7.2 ± 0.2 .

- N-[Tris(hydroxymethyl)methyl] glycine 1 117 mg
- Ethylenediamine disodium tetraacetate dehydrate 183 mg
- Magnesium acetate tetrahydrate 808 mg
- DL-dithiothreitol 6.7 mg
- Dextrin 25 000 mg
- Sucrose 925 mg
- Water 250 ml

6.16 ATP luminescent reagent, prepared from the following compositions. Once fully dissolved, let sit at room temperature for 15 min before use.

Use within 3 h of preparation. When a different ATP luminescent reagent is used, its composition shall be recorded.

— Luciferase (EC:1.13.12.7)	16.0 mg
— D-luciferin	12.6 mg
— Bovine serum albumin	56 mg
— Buffer solution for ATP luminescent reagent (6.15)	30 ml

6.17 ATP extracting reagent, prepared from the following compositions to be pH 7.2 $\pm$ 0.2.

When a different ATP extracting reagent is used, its composition shall be recorded.

— N-[Tris(hydroxymethyl)methyl] glycine	45 mg
— 10 % aqueous benzalkonium chloride	0.2 ml
— Water	9.8 ml

6.18 ATP eliminating reagent, prepared from the following compositions to be pH 6.0 $\pm$ 0.5. An agent to reduce the ATP in Tryptone soya broth (TSB) (**6.2**) or Nutrient broth (NB) (**6.5**) to less than 10^{-13} mol/L within 15 min.

— Apyrase (EC:3.6.1.5)	46 international units/ml
— Adenosine phosphate deaminase (EC:3.5.4.6 or EC:3.5.4.17)	46 international units/ml
— Sucrose	37 mg
— Bovine serum albumin	20 mg
— 0.05 mol/L buffer solution of 2-morpholinoethanesulfonicacid, monohydrate	10 ml

The reagent shall be used within 8 h of preparation.

When a different ATP eliminating reagent is used, its composition shall be recorded.

6.19 SCDLP or other medium for preparing ATP reference solution, prepared from the following compositions. An ATP reference solution should be prepared if the addition of neutralizing agents causes the ATP content in the shake-out solution to exceed 10^{-11} mol/L.

— SCDLP (6.8) or other medium	10 ml
— ATP eliminating agent (6.18)	1 ml

After mixing, maintain at 30 °C to 37 °C for 1 h to prevent microbiological contamination. Next, transfer to a hot-water bath (**5.3**) at 70 °C to 90 °C for 1 h and cool down to room temperature. Preserve the solution under refrigeration and use within 24 h.

6.20 Shake-out physiological saline Mix well the following compositions, then sterilize by autoclave (**5.28**).

— Sodium chloride (NaCl)	8.5 g
— Polysorbate 80	2.0 g
— Water	1 000 ml

NOTE 1 Reagents used in tests should be of analytical quality and/or suitable for microbiological purposes.

NOTE 2 Dehydrated products available on the commercial market are recommended for use in preparing the culture media. The manufacturer's instructions for the preparation of these products should be strictly followed.

7 Reference strains

7.1 Strains

The strain to be used shall be selected from Annex A.

7.2 Storage of strains

7.2.1 General

The strains shall be stored in accordance with the supplier's recommendations.

7.2.2 Ceramic bead method

Ceramic bead method shall be as follows.

- Obtain a sample of the freeze-dried bacterial strain following the recommendations supplied with the culture and resuspend it in 5 ml of Tryptone soya broth (TSB) (6.2). Obtain a sample of the suspension and isolate it in a Petri dish (5.17) containing Tryptone soya agar (TSA) (6.3).
- Incubate the cultures for 18 h to 24 h at $37\text{ °C} \pm 2\text{ °C}$. After incubation, use the culture isolated in the Petri dish to verify the purity of the strain. After verification, prepare the stock cultures.
- Sample 0.7 ml of the broth culture and spread it over the surface of the Petri dish containing the Tryptone soya agar (TSA). Incubate the culture on plates for 18 h to 24 h under the conditions specified in b).
- Add 10 ml of cryoprotective solution (6.13) to the surface of the TSA plate culture and resuspend the cells in the solution using a sterile glass spreader. Sample the suspended cells from the surface of the agar. Dilute them in 100 ml of cryoprotective solution and incubate for 30 min at 20 °C.
- Using a pipette (5.15), sample 1 ml of the suspension and transfer it to a cryogenic vial (5.16) containing the beads (5.19). Shake the vial in order to spread the suspended cells around the beads. Where a cryoprotective solution containing dimethylsulfoxide is used, do not let it stand longer than 1 min at ambient temperature. Where a cryoprotective solution containing glycerol is used, let it stand for 30 min at 20 °C.
- Withdraw the excess cryoprotective solution with a sterile pipette. Place the cryogenic vials in a freezer (5.12) set at -70 °C or lower.

- g) Carry out the quantitative measurement by plate count method specified in Annex C to confirm the number of bacteria. Prepare 10^{-6} and 10^{-7} dilutions of the suspension using the serial dilution method. Take a 1.0 ml sample of each dilution and transfer it to separate Petri dishes. Add 12 ml to 15 ml of nutritive solution, cooled down to $45\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$. Incubate for 18 h to 24 h under the conditions at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.
- h) Enumerate the plate cultures and confirm that the suspension contains less than 5×10^8 CFU/ml.

7.2.3 Glycerol suspension method

Glycerol suspension method shall be as follows.

- a) Inoculate a 15 ml culture tube containing 5 ml of appropriate medium with a freshly grown isolated colony. Incubate usually for 5 h to overnight at $37\text{ }^{\circ}\text{C}$ until the bacteria culture seems to reach the late logarithm or stationary phase in the growth curve.
- b) For each strain to be stored below $-70\text{ }^{\circ}\text{C}$, for the archives, prepare a sterile, labelled cryogenic vial. Place 225 μl of sterile 80 % glycerol in a cryogenic vial.
- c) Add 1.0 ml of the bacterial culture (frozen stock shall be 15 % glycerol). Mix well using the vortex mixer (5.4) and store in a tube at $-70\text{ }^{\circ}\text{C}$ or lower.
- d) For each strain to be stored at $-20\text{ }^{\circ}\text{C}$, as liquid glycerol working stock, pipette equal volumes of 80 % glycerol and bacterial culture into a labelled polypropylene tube. Mix the contents well to avoid formation of ice crystals that will decrease the viability of the cells. Place the tube in a freezer at $-20\text{ }^{\circ}\text{C}$. Check the viability of the cells after 1 week if possible.
- e) To recover a strain from the glycerol stock stored below $-70\text{ }^{\circ}\text{C}$, use a sterile toothpick to scrape pieces of the solid substance. Then streak the cells onto the appropriate medium. Do not thaw the frozen stock because each freeze-thaw cycle will result in a 50 % loss in cell viability.
- f) To use the $-20\text{ }^{\circ}\text{C}$ working stock, pipette 50 μl to 100 μl as inoculum for a 5 ml overnight culture.

7.2.4 Slant culture medium method

Slant culture medium method shall be as follows.

- a) For the preparation of slant culture medium, pour about 10 ml of Enumeration agar (EA) (6.11), dissolved by warming beforehand in a test tube, carry out autoclaved-sterilization by putting cotton stopper, after the sterilization is finished, place the test tube in clean room so as to incline against the horizontal surface by about 15° in angle and coagulate the contents. Dissolve that with the coagulated water lost and coagulate it again and use.
- b) The transference of bacteria shall be carried out according to the following procedure. Hold both the stock strain and slant agar to be transferred in one hand, hold the stem of platinum colony loop in the other hand and pull out the cotton stopper from test tube with this hand, and sterilize the mouth of the test tube with flame.

- c) Next, sterilize the platinum colony loop with flame, insert the loop into a part on slant agar having condensed water, cool it well and put it into a test tube of stock strain (see Figure 1). Scrape out the bacteria from the breeding surface of bacteria using the platinum colony loop, spread it on a slant agar, again sterilize the mouth of the test tube with flame, and place a cotton stopper as it was. Sterilize the platinum colony loop used with flame.
- d) Incubate the bacteria transferred to slant agar at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 24 h to 48 h, and thereafter preserve it at $5\text{ }^{\circ}\text{C}$ to $10\text{ }^{\circ}\text{C}$.

When spreading one platinum loop on the slant agar, disperse bacteria in condensed water as shown in Figure 1, and draw a straight line slant to get to the upper part. Separate once the point of platinum loop from culture medium, then again insert it into condensed water, and this time draw a zigzag line up to the upper part.

- e) Within one month from the transference, carry out the next transference of bacteria as same as previously to make passage culture. The passage culture, shall not be subcultured more than 10 times. When kept for one month or longer from the last transference, it shall not be used for the following transference.

NOTE : In the case of long term preservation, it may be preserved either by freeze drying or by being transferred on high layer culture medium and being layered with sterilized liquid paraffin.

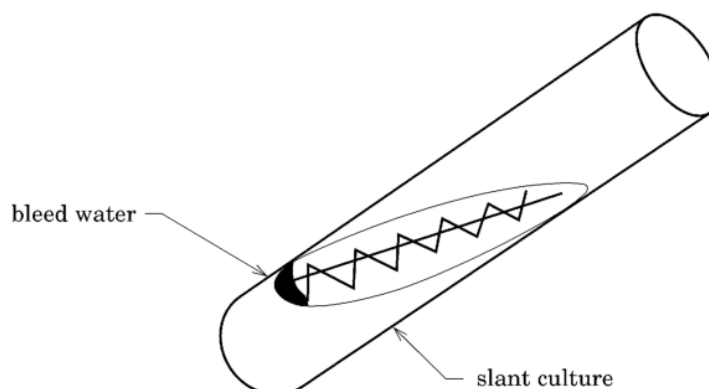


Figure 1 Transference of bacteria on slant culture medium

8 Test procedures

8.1 Absorption method

8.1.1 Incubation

8.1.1.1 Preincubation A

Pick up the preserved stock bacteria from the storage container using a platinum colony loop (5.7). Streak onto the plate of Enumeration agar (EA) (6.11) so that the colony after incubated is isolated. Incubate for 24 h to 48 h under the conditions at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$. The plate is kept at $5\text{ }^{\circ}\text{C}$ to $10\text{ }^{\circ}\text{C}$ and used within 1 week after the date of preparation.

The plate with colony taken once shall not be used again.

8.1.1.2 Preincubation B

Pour 20 ml of Nutrient broth (NB) (6.5) or Tryptone soya broth (TSB) (6.2) into a 100 ml Erlenmeyer flask with cap (5.20). Apply a platinum colony loop to pick one colony up from the incubation as specified in 8.1.1.1 and inoculate it in the broth. Incubate under the following conditions.

- Temperature: $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$
- Rate of shaking: 110 time/min and 30 mm width by reciprocal incubation shaker (5.27)
- Incubation time: 18 h to 24 h

8.1.1.3 Preincubation C

Pour 20 ml of Nutrient broth (NB) (6.5) or Tryptone soya broth (TSB) (6.2) into a 100 ml Erlenmeyer flask. Add 0.4 ml of the inoculum from the incubation as specified in 8.1.1.2 that contains 1×10^8 CFU/ml to 3×10^8 CFU/ml in bacteria concentration or a target ATP concentration of 1×10^{-6} mol/L to 3×10^{-6} mol/L to the flask and incubate under the following conditions.

- Temperature: $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$
- Rate of shaking: 110 time/min and 30 mm width by reciprocal incubation shaker
- Incubation time: $3\text{ h} \pm 1\text{ h}$

Target CFU or ATP concentration after incubation: 10^7 CFU/ml or 10^{-7} mol/L.

NOTE : The prepared inoculum is preserved by ice-cooling and used within 8 h.

8.1.2 Preparation of test inoculum

Adjust the bacteria after incubation in 8.1.1.3 to a concentration of 1×10^5 CFU/ml to 3×10^5 CFU/ml by a spectrophotometer or McFarland's nephelometer (5.1) using Nutrient broth (NB) (6.5) or Tryptone soya broth (TSB) (6.2) after it has been diluted 20 times with water at room temperature, or measure the ATP concentration of the bacteria after incubation in 8.1.1.3 by the luminescence method and adjust the ATP to a concentration of 1×10^{-9} mol/L to 3×10^{-9} mol/L.

Measure the bacteria concentration and the ATP concentration of prepared test inoculum according to the determination method specified in Annex C and Annex D, and take them as the concentration of test inoculum.

NOTE : The prepared inoculum is preserved by ice-cooling and used within 4 h.

8.1.3 Preparation and setting of test specimens

8.1.3.1 Preparation of test specimens

Obtain six test specimens and six control specimens with a mass of $0.40\text{ g} \pm 0.05\text{ g}$ and cut to a suitable size for test specimens.

NOTE : Three test specimens and three control specimens are used for zero time, immediately after inoculation. The remaining six specimens are used for the contact time after 18 h to 24 h incubation.