Investigative Research in Fiscal 2003
Commissioned by National Institute of Technology and Evaluation (NITE)

# Report: Establishment of Traceability and Estimation of Uncertainty in Evaluation Methods using Bacteria

(Summarized Version)

March 2004

Society of Industrial-Technology for Antimicrobial Articles (SIAA)

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# 1. Introduction

In December 1998, the "Advisory Board for Household Goods with Newly Developed Functions" of the Ministry of Economy, Trade and Industry (METI, former Ministry of International Trade and Industry, MITI) issued the Guidelines for Antibacterial Products. In response to the guidelines, JIS Z 2801:2000 "Antibacterial products-Test for Antibacterial activity and efficacy" was enacted. At the same time, Antibacterial testing was added as an accredited category in the Japan National Laboratory Accreditation system (JNLA) under the Industrial Standardization Law, and accreditation of related laboratories was started.

In accordance with the amendment of the international standards regarding the laboratory accreditation, which was from ISO/IEC Guide 25 to ISO/IEC 17025:1999, JNLA announced the policy to apply ISO/IEC 17025 [JIS Q 17025:2000] to all the laboratories as well as "requirements concerning uncertainty of the testing," from July 2001 for the new applications, and from January 2002 for the inspections of already-accredited laboratories.

The concept, "uncertainty of measurement," is unfamiliar in the laboratories, and the policies of each country have not been consolidated in the areas of electrical engineering and physics. Especially, bioassay that uses microorganisms is internationally considered to be one of the most difficult areas for applying the concepts; therefore it is necessary to provide examples that can be used as models to estimate the uncertainty for Antibacterial activity testing in the implementation of the laboratory accreditation system.

In the current context of increasing transfer of production and technology for the Antibacterial products in Japan to overseas areas such as Asia, Europe and the U.S., upgrading of the standards concerning Antibacterial products from national standards (JIS Z 2801:2000) to international standards (ISO standards) is desired both at home and abroad. Therefore, it is necessary to re-design the testing methods (i.e., ISO standardization of testing methods) that take uncertainty into account. In other words, in the global market, data provided by reliable laboratories that are accredited to meet the international standards are in demand, indicating the significant benefits of the system, in which data obtained in one laboratory are acceptable as valid worldwide, or "one-stop-testing."

In view of the future international standardization, as well as making the laboratory accreditation system in Japan conformable with ISO/IEC 17025, it was decided to carry out an investigative research study, funded with the "business contract services fee for the accredited laboratory" by the National Institute of Technology and Evaluation at the request of METI. As a part of the action, the committee for the "Investigative Research Regarding the Establishment of Traceability and Estimation of Uncertainty in the Evaluation Methods using Bacteria" (Uncertainty Committee) was organized in fiscal 2001, and the committee continued to be active in fiscal years 2002 and 2003.

The Society of Industrial-Technology for Antibacterial Articles (SIAA) was in charge of the investigative research in fiscal 2003 by the National Institute of Technology and Evaluation, and was reviewed in the Uncertainty Committee and Uncertainty Subcommittee.

This report describes the activity and the outcome of the "Investigative Research Regarding the Establishment of Traceability and Estimation of Uncertainty in the Evaluation Methods using Bacteria," which was carried out in fiscal years 2001, 2002 and 2003.

## 2. The Purpose of the Investigative Research

The "Estimation of uncertainty" in the area of bioassay, which is recognized internationally to be one of the most difficult areas for applying the concept, is under debate in the world, and unified understanding and utilization have not been brought to fruition. The supply of certified reference materials to obtain the traceability of measurement is still under preparation at home and abroad.

The JIS Z 2801:2000 is the standard for the evaluation method using bacteria, and the area of bioassay is internationally regarded as one of the most difficult areas for application of "establishment of traceability" and "estimation of uncertainty." Therefore, the objective of this study is to take the initiative work in investigation and examination regarding these concepts, and proceed to "production of the standard reference materials," which are necessary for the examinations, in order to promote the internationalized laboratory accreditation system and prepare for the future international standardization process.

Production of homogeneous, stable reference materials enables the designing of testing methods that take uncertainty into account, which is data-based, logical, assuring, and internationally acceptable (revision of JIS Z 2801 to conform to the international standards). It also enables requiring the estimation of uncertainty by using the reference materials as control samples for the proficiency tests and for use within the laboratory in the management of a laboratory accreditation system. Although the production of reference materials in the area of biology is regarded as extremely difficult, the effectiveness is considered to be significant. Therefore, it will be highly meaningful to aim for the production of reference materials and international standards by utilizing the knowledge of biotechnology and nanotechnology in Japan based on the JIS Z 2801 as a case study.

## 3. Selection of Standard Testing Materials

Two types of materials were selected based on the results of the investigative report in fiscal 2003.

(1) Silver Acrylic Coated Film

The results for the investigative research in fiscal years 2001 and 2002 show that, although silver acrylic coated film has a certain extent of problems of inconsistency in Antibacterial activity between productions, the silver acrylic coated film is highly useful as a standard testing material due to its mass production capability and low production cost; therefore it can also be used as a quality control sample for everyday testing. The goal of this fiscal year is to complete the production of the standard testing materials with a stable quality by using a revised production method.

(2) GaAs Wafer

According to the results of the investigative report in fiscal 2002, the Antibacterial activity for this material was 2 to 3, which is an ideal range of values for use as a standard testing material. Moreover, GaAs meets the requirement for use as a standard testing material because of the readiness in availability by virtue of a stable mass production, and under the high level of quality control and supply in the semiconductor market, despite the fact that GaAs wafer is a high purity material.

# 4. Laboratories that Performed Antibacterial Testing and the Test Conditions

## 4.1 Laboratories that Performed the Testing

The below five laboratories were in charge of the Antibacterial testing. All of these laboratories are accredited by JNLA.

- INAX Corporation (INAX)
- Ishizuka Glass Co., Ltd.
- Sumitomo Osaka Cement Co., Ltd.
- Japan Food Research Laboratories (JFRL)
- Japan Synthetic Textile Inspection Institute Foundation (KAKEN)

## 4.2 Testing Conditions

The tests using GaAs wafer and silver acrylic coated film were performed under the conditions described below.

(1) Testing Method

In accordance with JIS Z 2801, detailed "Antibacterial testing procedures (hereinafter referred to as procedures)," which were adapted to the properties of each standard testing material, were prepared, and the testing was performed based on these procedures.

(2) Bacteria for Testing (the common strain)

*Staphylococcus aureus* (NBRC 12732) were ordered by JFRL to the microbial resource preservation center (National Institute of Technology and Evaluation Biological Resource Center, or NBRC). The strain was distributed to each laboratory, and then the strain was reconstructed for use by each laboratory. All the laboratories performed the Antibacterial testing several times at the same time period; thus the same passage number of the strain was used in all the laboratories. (The strain passaged was performed in each laboratory.)

(3) Culture Media and Culture Containers

The following culture media and cover films, which are considered to affect the testing performance, were purchased collectively by JFRL and distributed to each laboratory:

[1] Standard methods agar medium (Eiken Chemical Co., Ltd., 300 grams)

[2] Standard methods broth medium (Eiken Chemical Co., Ltd., 100 grams)

[3] Cover films (Stomacher® bags, Organo Corporation): the film was cut into pieces of the desired size at each laboratory aseptically.

[4] Culture containers (Jallee Co., Ltd., No. 5 airtight boxes): The containers that had been used in the investigative research in fiscal 2002 were used continuously. (The identical containers were provided by INAX to KAKEN.)

# 5. Evaluation of PET Film with Varnish Coating Containing Water Soluble Silver Antibacterial Agent (Silver Acrylic Coated Film


## Summary

In October and November of 2003, two sets of testing of reproducibility were carried out with silver acrylic coated film, which were prepared on September 5. The results for the two tests show that the Antibacterial activity values for the samples with 350 ppm ranged 1.8 to 2.0, and those with 450 ppm ranged 2.3 to 2.7.

ANOVA was used to analyze the results, which were from the four laboratories, because the data from one laboratory were considered unreliable in the examination with z-scores. The results of ANOVA show that reproducibility and laboratory were not statistically significant, suggesting that the use of a stable quality of samples in the testing leads to results that were consistent among the laboratories.

Expanded uncertainty (i.e., uncertainty of the testing method) was 0.91 (with k = 2.01), calculated based on the variance in the reproducibility test of the four laboratories.

It was evaluated that the samples in this production lot were sufficiently useful as standard testing samples.


## 5.1 Components of Silver Acrylic Coated Film

The components for the production of the standard testing material are the same as those used in the investigative research in fiscal 2002.

### 5.1.1 Coating Agent

The composition of the coating agent used for the preparation of the standard testing material is described as follows:

(1) Antibacterial agent: TSC-N, Meiji Dairies Corporation (o-mercaptobenzoato silver (1) sodium-hydrate oligomer)

(2) Polymer: polyacrylic alkyl ester/neutralized ammonium of methacrylic acid copolymer

(3) Additive: diacetylene containing surfactant 1%

(4) Solvent: water

### 5.1.2 Type and Properties of the Polymer (Cited from the investigative report in fiscal 2002)

(1) Composition, Molecular Weight, Non-Volatile Components, Solvents and Viscosity

[1] Composition: Polyacrylic alkylester/ methacrylic acid copolymer

[2] Molecular weight: emulsion polymerization (it was not measurable with tetrahydrofuran (THF) solution or Portable Gas Chromatograph; it is estimated that it is more than several millions)

[3] Non-volatile components: 22.5 ± 1.0% (residue after drying 150°C/20 min)

[4] Solvent: water

[5] Viscosity: 1500 ± 500 cps (BM type viscometer #3/12 rpm at 25°C)

(2) Swelling Property

When contact with bacterial culture occurs, it swells and releases an Antibacterial agent immediately.

(3) Base Types and the Effectiveness

Maintaining water-solubility by the neutralizing effect of ammonium:
ammonium of methacrylic acid ammonium salt is partially decomposed to methacrylic acid in the drying process after the coating application, decreasing the water solubility.

(Because it was not possible to make the coating thickness thin, this is an attempt to minimize the effect of inconsistent thickness by controlling the release speed of the Antibacterial agent and the elution speed under the swelling conditions. Therefore, if the coating is removed by washing, the amount of Antibacterial agent is subject to change.

(4) pH

7.5 ± 0.5 (at 25°C)

(5) Residual Monomers related (pertinent) to Polymers

| | |
|---|---|
| Ethyl acrylate monomer | not detected (20 ppm or less) |
| Methyl methacrylate monomer | not detected (20 ppm or less) |
| Methacrylic acid monomer | 850 ppm |

### 5.1.3 Resistance against Electron Beam (Cited from the Investigative Report in 2001)

A sample with 400 ppm of Antibacterial agent was irradiated with a 60 kGy-electron beam to examine the effect on the Antibacterial activity. No effect was observed on the viable counts under the condition of sterilization treatment using a 60 kGy-electron beam.

## 5.2 Preparation and Specifications of the Standard Testing Materials

The preparation conditions and specifications for the standard testing material in this study are as follows:

(1) Coating agent: refer to 5.1.1.

(2) Antibacterial agent concentration: 0, 300, 350, 400, 450, 500, 550, and 600 (ppm)

(3) Coating thickness: 5 ± 1 μm

(4) Base material: PET film, Nisshinbo Industries (E-5101); matt-finished back side, 50 μm film thickness

(5) Conditions for the coating application

Coating method: Micro Gravure™ (50 lines)
Drying conditions: the first oven: 140°C/10 Hz; the second oven: 140°C/10 Hz
Coating speed: 3 m/min

(6) Preparation date: September 5, 2003

### 5.2.1 Examination of Preparation Conditions

#### 5.2.1-1 Condition Settings for the Coating Test (August 1, 2003)

The results for the Antibacterial activity testing using the materials made on August 1, 2003, were not successful (the viable counts for the control samples after 24 hours of inoculation were less than ($1 \times 10^4$)). The ovens used for preparation were jet nozzle type ovens, which have a higher flow of heated air; therefore, the surface of the coated materials tends to be dried rapidly. As a result, ammonium in the bottom layer of the Antibacterial coating was unlikely to be dried until the normal condition.

#### 5.2.1-2 Condition Settings for the Coating Test (August 21, 2003)

In this attempt, the air flow setting was decreased to 10 Hz.

Table 1 Condition Settings for the Coating Test

| Sample No. | Antibacterial agent concentration (ppm) | Oven temperature (°C) | | Material temperature (°C) | Air flow (Hz) | Water rubbing (# of times) | Ammonium (measured on August 26) |
|---|---|---|---|---|---|---|---|
| | | First | Second | | | | |
| A1 | 0 | 100 | 100 | 67 | 10/10 | 5 | |
| A2 | 0 | 100 | 125 | 75 | 10/10 | 8 | |
| A3 | 0 | 100 | 140 | 84 | 10/10 | 10 | |
| A4 | 0 | 120 | 140 | 87 | 10/10 | 40 | 0.100 |
| A5 | 0 | 140 | 140 | 89 | 10/10 | 75 | 0.079 |
| A6 | 0 | 140 | 160 | 99 | 10/10 | >100 | 0.058 |
| A7 | 350 | 120 | 140 | 89 | 10/10 | 90 | |
| A8 | 350 | 140 | 140 | 91 | 10/10 | 108 | |
| A9 | 350 | 140 | 160 | 98 | 10/10 | >200 | |
| A10 | 400 | 120 | 140 | 91 | 10/10 | 40 | 0.090 |
| A11 | 400 | 140 | 140 | 91 | 10/10 | 43 | 0.085 |
| A12 | 400* | 140 | 140 | 91 | 10/10 | 95 | 0.053 |
| A13 | 400 | 140 | 160 | - | 10/10 | 130 | 0.057 |

Antibacterial agent lot: C25; Coating agent NV: 15%; Reverse: 200%
* Coating speed of 2 m/min was used

Table 2 Results of the Antibacterial Activity Testing for the Testing Material Samples with Different Condition Settings

| Sample No. | Viable counts | | | | | | | | Antibacterial activity |
|---|---|---|---|---|---|---|---|---|---|
| | Immediately after inoculation | | | | 24 hours after inoculation | | | | |
| | n-1 | n-2 | n-3 | Mean | n-1 | n-2 | n-3 | Mean | |
| A4 | 2.0E+5 | 1.9E+5 | 2.0E+5 | 2.0E+5 | 1.2E+5 | 1.0E+5 | - | 1.1E+5 | - |
| A5 | 2.0E+5 | 1.8E+5 | 1.9E+5 | 1.9E+5 | 1.2E+5 | 9.5E+4 | - | 1.1E+5 | - |
| A6 | 2.1E+5 | 2.0E+5 | 2.0E+5 | 2.0E+5 | 1.1E+5 | 1.1E+5 | - | 1.1E+5 | - |
| A7 | - | - | - | - | 1.4E+3 | 1.1E+2 | - | 7.6E+2 | 2.2 |
| A10 | - | - | - | - | 4.0E+1 | 2.0E+2 | - | 1.2E+2 | 3.0 |
| A11 | - | - | - | - | 8.0E+1 | 6.0E+4** | - | 8.0E+1 | 3.1 |
| A12 | - | - | - | - | 1.4E+2 | 1.1E+2 | - | 1.3E+2 | 2.9 |
| A13 | - | - | - | - | 5.0E+1 | 1.0E+2 | - | 7.5E+1 | 3.2 |

Inoculation counts: $3.6 \times 10^5$
** Omitted as an outlier

The results indicate the Antibacterial activity values fall in the range of the expected values under the conditions used in this test. Therefore, it was determined that the condition settings listed in Table 1 for the preparation of the standard testing materials would be used.

### 5.2.1-3 Preparation of the Standard Testing Materials for Evaluation (September 5, 2003)

Table 3 Conditions for the Preparation of Standard Testing Material for Evaluation

| Sample No. | Antibacterial agent concentration (ppm) | Oven temperature (°C) | | Material temperature (°C) | Air flow (Hz) | Water rubbing (# of times) | Ammonium (measured on September 9) |
|---|---|---|---|---|---|---|---|
| | | First | Second | | | | |
| B1 | 300 | 140 | 140 | 89 | 10/10 | 37 | |
| B2 | 350 | 140 | 140 | 90 | 10/10 | 38 | 0.054 |
| B3 | 400 | 140 | 140 | 92 | 10/10 | 47 | 0.079 |
| B4 | 450 | 140 | 140 | 92 | 10/10 | 39 | |
| B5 | 500 | 140 | 140 | 91 | 10/10 | 50 | |
| B6 | 550 | 140 | 140 | 91 | 10/10 | 35 | |
| B7 | 600 | 140 | 140 | 91 | 10/10 | 79 | |
| B8 | 0 | | 140 | 91 | 10/10 | 44 | 0.071 |

Antibacterial agent lot: C25; Coating agent NV: 15%; Reverse: 200%

Table 4 Results for the Antibacterial Activity Testing of the Standard Testing Materials for Evaluation

| Sample No. | Viable counts | | | | | | | | Antibacterial activity |
|---|---|---|---|---|---|---|---|---|---|
| | Immediately after inoculation | | | | 24 hours after inoculation | | | | |
| | n-1 | n-2 | n-3 | Mean | n-1 | n-2 | n-3 | Mean | |
| B8 | 5.7E+5 | 5.0E+5 | 4.3E+5 | 5.0E+5 | 2.7E+5 | 1.5E+5 | - | 2.1E+05 | — |
| B2 | - | - | - | - | 1.2E+3 | 9.5E+2 | - | 1.1E+03 | 2.3 |
| B3 | - | - | - | - | 6.5E+2 | 9.1E+2 | - | 7.8E+02 | 2.4 |

Inoculation counts: $6.0 \times 10^5$

#### 5.2.1-4 Summary

As shown in Tables 1 and 2, the results show that stable levels of Antibacterial activity values are obtained in a wide range of oven temperature settings, from 120/140 to 140/160.

The results also indicate that the measurements of residual ammonium with the ATR method are accurate and precise, and it was determined that they can be usable as an item for quality control, and that the control range for this case would be 0.054 to 0.101.

### 5.3 Test for Uniformity of Coating

To evaluate the uniformity of the standard samples, the amount of silver, which is a component of the Antibacterial agent, was selected as an indicator, and the amount of silver per sample piece (40 × 40 mm) was measured.

#### 5.3.1 Samples

The evaluation was carried out using the standard testing material samples listed in Table 3 on September 5, 2003; the concentration and the number of samples are:

[1] Five samples of 350 ppm (n = 5)

[2] Five samples of 450 ppm (n = 5)

#### 5.3.2 Sampling Method

Two sets of the standard testing material samples were randomly selected out of the total ten sets stored, and five out of the eight sample pieces contained in the selected two sets were randomly selected for use as evaluation samples.

#### 5.3.3 Theoretical Estimated Values of Silver

[1] 350 ppm samples: 1.2 μg/ sample piece (16 $cm^2$)

[2] 450 ppm samples: 1.4 μg/ sample piece (16 $cm^2$)

The equation used for the calculation is:
amount of silver (μg) = amount of coating (g) × concentration (ppm) × 0.95 × 107.9/301

#### 5.3.4 Measurement of Silver

The amount of silver contained per sample piece, of which there are a total of five pieces of each of the standard testing materials with 350 ppm and 450 ppm, was measured using atomic absorption spectrophotometry.

(1) Preparation of the sample solutions

Each sample piece was placed in a Kjeldahl flask, and 10 ml of nitric acid was added and slowly heated. When the strong reaction ended, 5 ml of sulfuric acid was added, and the sample was reheated.

When the solution started to become dark-colored, nitric acid was added 2 ml at a time, and the solution was heated until it turned almost clear. The solution was continuously heated until white fumes appeared, and then was removed from the heat. After it cooled, the solution was poured into a volumetric flask and the solution sample was ready.

(2) Testing Method

A part or all of the sample solution, 200 ml, was measured into a separatory funnel, and 10 ml of 50% di-ammonium hydrogen citrate and two drops of bromthymol blue were added and then neutralized with ammonium hydroxide, and water was added to make about 100 ml of solution. Next, 10 ml of 10% DDTC solution was added and mixed and set aside; after 5 minutes, precisely 10 ml of methyl isobutyl ketone was added and agitated for 5 minutes. After letting it stand, the methyl isobutyl ketone layer was fractionated, and the absorbance was measured with the atomic absorption spectrophotometry. The standard silver solutions of 0.25 ppm, 0.5 ppm and 1.0 ppm were prepared from the standard reference solution for silver, 10 ml of each solution was precisely measured into a 200 ml-separatory funnel, 40 ml of water and 10 ml of 50% di-ammonium hydrogen citrate and two drops of bromthymol blue were added, and the same preparation process as for the sample solution was repeated. The silver concentration levels in the samples were calculated using the calibration curve that was created based on the obtained absorbance values.

(3) Conditions for the measurement with atomic absorption spectrophotometry

- Model: AA-890 (Nippon Jarrell-Ash Co., Ltd.)
- Light source: silver hollow cathode lamp (Hamamatsu Photonics K.K.)
- Wavelength measured: 328.1 nm
- Flame: air-acetylene

### 5.3.5 Results for Silver Measurements

Table 5 Results for Silver Measurements and Sample Weight

| | 350 ppm | | 450 ppm | |
|---|---|---|---|---|
| Sample No. | Sample weight (g/piece) | Ag weight (μg/piece) | Sample weight (g/piece) | Ag weight (μg/piece) |
| 1 | 0.1205 | 0.90 | 0.1190 | 1.34 |
| 2 | 0.1213 | 1.02 | 0.1188 | 1.02 |
| 3 | 0.1205 | 0.83 | 0.1201 | 1.15 |
| 4 | 0.1204 | 0.96 | 0.1193 | 1.09 |
| 5 | 0.1201 | 0.82 | 0.1193 | 1.31 |
| Mean | 0.1206 | 0.91 | 0.1193 | 1.18 |
| SD | | 0.09 | | 0.14 |

The amount of silver was measured for each piece of the samples, which are five pieces each of the standard testing materials with 350 ppm and 450 ppm. The mean for the 350 ppm group is 0.91 μg/piece, the standard deviation is 0.09 μg/piece, the coefficient of variation is 9.4%; the mean for the 450 ppm group is 1.18 μg/piece, the standard deviation is 0.14 μg/piece, and the coefficient of variation is 11.8%, indicating that the samples within the same group are homogeneous.

## 5.4 Preliminary Test: Determination of Silver Concentration Levels used for the Testing

(1) Preliminary Test (performed at laboratories B and E)

The Antibacterial activity of the samples for the standard testing materials (with the silver concentration levels of 300 ppm, 350 ppm, 400 ppm, 450 ppm, 500 ppm, 550 ppm, and 600 ppm; prepared in September 2003) were evaluated with a method in which the samples were kept in an inverted position, in accordance with JIS Z 2801 (one of the surface sides of the samples was not wiped with ethanol).

(2) The results for the preliminary tests carried out by the two laboratories are shown in Table 6.

Table 6 Preliminary Test

| | | | | | Laboratory | | E | |
|---|---|---|---|---|---|---|---|---|
| [1] Common Strain (*Staphylococcus aureus* NBRC12732) | | | | | | | | |
| Sample | No. | Times dilution | Counts | Viable counts (cfu/sample) | Viable counts (mean) | Viable counts (log) | Anti-microbial activity | Anti-microbial activity (mean) |
| Blank control film (immediate) [1] | 1 | 2 | 282 | 2.8E+5 | | 5.45 | | |
| | 2 | 2 | 309 | 3.1E+5 | 3.0E+5 | 5.49 | | |
| Blank control film (24 hrs) [2] | 1 | 3 | 135.5 | 1.4E+6 | | 6.13 | | |
| | 2 | 3 | 208 | 2.1E+6 | 1.7E+6 | 6.32 | | |
| 0 ppm | 1 | 1 | 253 | 2.5E+4 | | 4.40 | 1.83 | |
| | 2 | 1 | 301 | 3.0E+4 | 2.8E+4 | 4.48 | 1.76 | 1.79 |
| 300 ppm | 1 | 1 | 317.5 | 3.2E+4 | | 4.50 | 1.73 | |
| | 2 | 1 | 129.5 | 1.3E+4 | 2.2E+4 | 4.11 | 2.12 | 1.89 |
| 350 ppm | 1 | 1 | 95.5 | 9.6E+3 | | 3.98 | 2.25 | |
| | 2 | 1 | 117 | 1.2E+4 | 1.1E+4 | 4.07 | 2.17 | 2.21 |
| 400 ppm | 1 | 0 | 326.5 | 3.3E+3 | | 3.51 | 2.72 | |
| | 2 | 1 | 300.5 | 3.0E+4 | 1.7E+4 | 4.48 | 1.76 | 2.01 |
| 450 ppm | 1 | 0 | 122.5 | 1.2E+3 | | 3.09 | 3.15 | |
| | 2 | 0 | 144.5 | 1.4E+3 | 1.3E+3 | 3.16 | 3.08 | 3.11 |
| 500 ppm | 1 | 0 | 123.5 | 1.2E+3 | | 3.09 | 3.14 | |
| | 2 | 0 | 296 | 3.0E+3 | 2.1E+3 | 3.47 | 2.76 | 2.91 |
| 550 ppm | 1 | 0 | 215.5 | 2.2E+3 | | 3.33 | 2.90 | |
| | 2 | 0 | 165.5 | 1.7E+3 | 1.9E+3 | 3.22 | 3.02 | 2.96 |
| 600 ppm | 1 | 0 | 52 | 5.2E+2 | | 2.72 | 3.52 | |
| | 2 | 0 | 51 | 5.1E+2 | 5.2E+2 | 2.71 | 3.53 | 3.52 |

| | | | | | Laboratory | | B | |
|---|---|---|---|---|---|---|---|---|
| [1] Common strain (*Staphylococcus aureus* NBRC12732) | | | | | | | | |
| Sample | No. | Times dilution | Counts | Viable counts (cfu/piece) | Viable coutns (mean) | Viable couts (log) | Anti-microbial activity | Anti-microbial activity (mean) |
| Blank cntrol film (immediate) [1] | 1 | 2 | 212 | 2.1E+5 | | 5.33 | | |
| | 2 | 2 | 222 | 2.2E+5 | 2.2E+5 | 5.35 | | |
| Blank control film (24 hrs) [2] | 1 | 3 | 351.5 | 3.5E+6 | | 6.55 | | |
| | 2 | 3 | 278.5 | 2.8E+6 | 3.2E+6 | 6.44 | | |
| 0 ppm | 1 | 1 | 275.75 | 2.8E+4 | | 4.44 | 2.06 | |
| | 2 | 2 | 39.5 | 4.0E+4 | 3.4E+4 | 4.60 | 1.90 | 1.97 |
| 300 ppm | 1 | 2 | 53 | 5.3E+4 | | 4.72 | 1.77 | |
| | 2 | 2 | 42 | 4.2E+4 | 4.8E+4 | 4.62 | 1.88 | 1.82 |
| 350 ppm | 1 | 1 | 320.5 | 3.2E+4 | | 4.51 | 1.99 | |
| | 2 | 1 | 174 | 1.7E+4 | 2.5E+4 | 4.24 | 2.26 | 2.11 |
| 400 ppm | 1 | 1 | 64 | 6.4E+3 | | 3.81 | 2.69 | |
| | 2 | 1 | 89.5 | 9.0E+3 | 7.7E+3 | 3.95 | 2.55 | 2.61 |
| 450 ppm | 1 | 0 | 128 | 1.3E+3 | | 3.11 | 3.39 | |
| | 2 | 0 | 115.5 | 1.2E+3 | 1.2E+3 | 3.06 | 3.44 | 3.41 |
| 500 ppm | 1 | 0 | 61.5 | 6.2E+2 | | 2.79 | 3.71 | |
| | 2 | 0 | 78 | 7.8E+2 | 7.0E+2 | 2.89 | 3.61 | 3.65 |
| 550 ppm | 1 | 0 | 248 | 2.5E+3 | | 3.39 | 3.10 | |
| | 2 | 0 | 145.5 | 1.5E+3 | 2.0E+3 | 3.16 | 3.34 | 3.20 |
| 600 ppm | 1 | 0 | 86 | 8.6E+2 | | 2.93 | 3.56 | |
| | 2 | 0 | 84.5 | 8.5E+2 | 8.5E+2 | 2.93 | 3.57 | 3.57 |

Notes: [1] Viable counts immediately after inoculation
[2] Viable counts 24 hours after inoculation

The results of the two laboratories were in agreement each other. It is also observed that there is dependence between the Antibacterial activity values and the amount of silver. Accordingly, it was determined that the preparation of the standard testing materials was successful and that the evaluation testing would be carried out with these standard samples. It was also decided that two levels of silver concentration, 350 ppm and 450 ppm, which yielded the Antibacterial activity values ranged 2 to 3, respectively, would be used for the evaluation testing.

## 5.5 Antibacterial Activity Testing

### 5.5.1 Standard Samples

[1] Samples with no treatment (0 ppm)

[2] Samples with Antibacterial agent coating (350 ppm and 450 ppm)

Stomacher® bags (polyethylene, PE) were used as blank control samples.

The samples were placed in small polyethylene bags, separated by concentration levels, and the bags were placed in a light shield aluminum bag, and were distributed to the laboratories.

### 5.5.2 Testing Method

The silver coated film samples, which were from the same production lot, distributed from the place where they were prepared, were stored at each laboratory. Using these samples, two sets of testing were carried out, the first test in the early October 2003, and the second test in the early November 2003.

### 5.5.3 Testing Results

Table 7 The z-scores for the Antibacterial Activity Values (First Test)

| Sample | Laboratory | z-score | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 350 ppm | A | 1.17 | 0.86 | 1.14 |
| | B | 0.75 | 0.52 | 0.75 |
| | C | 0.37 | 0.83 | 0.18 |
| | D | 1.76 | 2.37 | 2.00 |
| | E | 0.00 | 0.11 | 0.03 |
| 450 ppm | A | 0.72 | 0.74 | 0.54 |
| | B | 0.25 | 0.94 | 0.67 |
| | C | 0.57 | 0.47 | 0.59 |
| | D | 3.61 | 0.91 | 1.32 |
| | E | 0.00 | 0.45 | 0.14 |

Table 8 The z-score for the Antibacterial Activity Values (Second Test)

| Sample | Laboratory | z-score | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 350 ppm | A | 0.43 | 0.48 | 0.69 |
| | B | 0.00 | 0.32 | 0.00 |
| | C | 0.03 | 0.03 | 0.05 |
| | D | 2.56 | 2.54 | 5.13 |
| | E | 1.39 | 1.10 | 1.23 |
| 450 ppm | A | 0.57 | 0.91 | 0.47 |
| | B | 0.62 | 0.62 | 0.00 |
| | C | 1.10 | 0.93 | 0.83 |
| | D | 1.53 | 1.45 | 0.67 |
| | E | 0.67 | 0.59 | 0.05 |

Table 9 Means and Standard Deviations of the Antibacterial Activities (First Test)

| Sample | Mean | SD |
|---|---|---|
| All the 5 laboratories | | |
| Ag-coated film 350 ppm | 1.99 | 0.69 |
| Ag-coated film 450 ppm | 2.63 | 0.92 |
| | | (N = 15) |
| 4 laboratories (Lab D is not included) | | |
| Ag-coated film 350 ppm | 1.72 | 0.42 |
| Ag-coated film 450 ppm | 2.29 | 0.45 |
| | | (N = 12) |

Table10 Means and Standard Deviations of the Antibacterial Activities (Second Test)

| Sample | Mean | SD |
|---|---|---|
| All the 5 laboratories | | |
| Ag-coated film 350 ppm | 2.02 | 0.59 |
| Ag-coated film 450 ppm | 2.69 | 0.70 |
| | | (N = 15) |
| 4 laboratories (Lab D is not included) | | |
| Ag-coated film 350 ppm | 1.78 | 0.26 |
| Ag-coated film 450 ppm | 2.42 | 0.41 |
| | | (N = 12) |

First, results of all the five laboratories were examined. The results showed that the z-scores were greater than 2 from one laboratory, which were considered as unreliable values; therefore, the detailed analysis was performed using the results from the other four laboratories.

In this set of testing, the Antibacterial activity testing was replicated twice by four laboratories, using two sorts of test pieces with repeated measurement of three samples. Because the data could not be randomized between the replicated tests or between the laboratories, this test was regarded as split-plot experiments. Accordingly, the data were analyzed with Analysis of Variance (ANOVA) using the split-plot approach. The summary of the results of ANOVA and the calculation results of uncertainty are shown below.

Table11 ANOVA Table and Uncertainty

| Sources of variation | SS | Df | V | F ratio | F (p = 0.05) | F (p = 0.01) | F test |
|---|---|---|---|---|---|---|---|
| Reproducibility [R] | 0.1200 | 1 | 0.1200 | 0.40 | 10.13 | 34.12 | |
| Laboratory [L] | 4.6569 | 3 | 1.5523 | 5.18 | 9.28 | 29.46 | |
| R × L (error term 1: $e_1$) | 0.8991 | 3 | 0.2297 | 13.84 | 2.90 | 4.46 | ** |
| Sample piece [P] | 4.4408 | 1 | 4.4408 | 205.12 | 4.15 | 7.50 | ** |
| L × P | 0.3887 | 3 | 0.1296 | 5.98 | 2.90 | 4.46 | ** |
| R × P | 0.0133 | 1 | 0.0133 | 0.62 | 4.15 | 7.50 | |
| R × L × P | 0.1160 | 3 | 0.0387 | 1.79 | 2.90 | 4.46 | |
| Error term 2 ($e_2$) | 0.6928 | 32 | 0.0217 | | | | |
| Total | 11.3277 | 47 | | | | | |

Notes: [1] SS: sum of squares; Df: degrees of freedom; V: mean square
[2] **: significant at 1% level

As shown in Table11, the main-plot error ($e_1$:first order error) of R×L indicates $e_1$ is highly significant at 1% level against the second error $e_2$ (error for replication of measurement ). On the other hand, there is no statistical significance seen for R×P and R×L×P against $e_2$, and so effects of these two are pooled into sub-plot error ($e_2$'). Table12 shows the result of analysis of variance after pooling the non-significant variations.

Table12 ANOVA Table and Uncertainty (non-significant variations were included in $e_2$')

| Sources of variation | SS | Df | V | F ratio | F (p = 0.05) | F (p = 0.01) | F test |
|---|---|---|---|---|---|---|---|
| Reproducibility [R] | 0.1200 | 1 | 0.1200 | 0.40 | 10.13 | 34.12 | |
| Laboratory [L] | 4.6569 | 3 | 1.5523 | 5.18 | 9.28 | 29.46 | |
| R × L (error term 1: $e_1$) | 0.8991 | 3 | 0.2997 | 13.84 | 2.90 | 4.46 | ** |
| Sample piece [P] | 4.4408 | 1 | 4.4408 | 194.46 | 4.11 | 7.50 | ** |
| L × P | 0.3887 | 3 | 0.1296 | 5.67 | 2.87 | 4.46 | ** |
| Error term 2 ($e_2$') | 0.8221 | 36 | 0.0228 | | | | |
| Total | 11.3277 | 47 | | | | | |

Reproducibility (R) and laboratory (L) were not significant.

The estimated variances are σe1^2 for the error (term) 1, σe1^2 + 12σL^2 for laboratory (L),
σe1 = $\sqrt{0.2997}$ = 0.548, σL = $\sqrt{\{(1.5523 - 0.2997)/12\}}$ = 0.323.

Combined standard uncertainty (UC) is calculated by taking into account the variance components, laboratory (L) and error (term) 1 ($e_1$), and the number of times of repetition was 3; therefore,
UC = $\sqrt{(\sigma L\text{^}2 + \sigma e1\text{^}2/3)}$ = $\sqrt{(0.323\text{^}2 + 0.2997/3)}$ = 0.452.

Expanded uncertainty with the t-value 2.01 (for Df = 47 at α = 0.05) is
0.452 × 2.01 = 0.91.

# 6. Evaluation of GaAs Wafer

## Summary

(1) Condition for GaAs Wafer and Antibacterial Activity

[1] The microbial activities increase by the removal and reapplication of GaAs wafer, but expanded uncertainty (U) also increases.

[2] The difference in microbial activities between the samples with no-tilt and 2° tilt-off is less significant compared to the difference in the presence or absence of the removal and reapplication process of the Antibacterial treatment.

(2) Effect of Light Illumination on the GaAs Wafer and Antibacterial Activities

Light illumination possibly increases the microbial activities of GaAs wafer. However, this is the case where the intensity of light is considerably high, and the effect of the intensity of light in a regular laboratory environment is not known.

(3) Concentration of the Eluted Components from GaAs Wafer and Antibacterial Activities

No correlation was observed between the concentration levels of gallium and arsenic eluted from the GaAs wafer and the Antibacterial activities.

(4) The testing was carried out using sample D, in a manner so that the testing conditions (i.e., light intensity and degree of surface oxidation) are as close as possible between the five laboratories, and the results show the Antibacterial activity is 2.75, the standard deviation is 1.20, and the expanded uncertainty is 3.48. Regardless of the fact that the testing conditions were made uniform, the variation between the laboratories was great.

(5) The results for the repetitive testing also show some variation within the same laboratory.

(6) Further examinations are necessary to clarify the mechanisms of Antibacterial activities on GaAs wafers and to determine if there are any factors that cause biases depending on the laboratories in the current testing method for GaAs wafers.

## 6.1 Specifications of GaAs Wafer

GaAs wafers are plate-shaped solids composed of over 99.9% gallium arsenide (51.8% as metallic arsenic), and are physicochemically compound monocrystals, which have a homogeneous crystal structure. The specifications for the GaAs wafers that were used in the evaluation testing in this fiscal year are as follows.

(1) Crystal growth method: VB (Vertical Bridgman) method

(2) Conduction type: semi-insulating

(3) Dopant: not added

(4) Etch pit density (EPD): ≤10,000 $cm^{-2}$

(5) Resistivity: $5 \times 10^7$ to $4 \times 10^8$ Ω · cm

(6) Electron mobility: ≥3,000 $cm^2/V \cdot s$

(7) Crystallographic orientations:

[1] Specification A: (100) ± 0.30° (the products with no tilt: used as samples A and D)

[2] Specification B: (100) 2° tilt-off <110> ± 0.3°: the products with 2° tilt-off: used as sample C)

(8) Surface finish: mirror finish on both sides

(9) Final cleaning: super clean

(10) Flatness: Warp ≤5 μm

(11) Package: wafer tray, N2 pack

(12) Thickness: 625 ± 25 μm

(13) Diameter: 100.0 ± 0.3 mm (4"), 76.0 ± 0.3 mm (3")

(14) Edge rounding: 0.25 mmR

(15) Primary flat lengths: 32.5 ± 1.0 mm (4"), 22.0 ± 1.0 mm (3")

(16) Secondary flat lengths: 18.0 ± 1.0 mm (4"), 12.0 ± 1.0 mm (3")

(17) Locations of primary and secondary flat lengths: European Japanese flat (or US flat)

## 6.2 Antibacterial Activity test for GaAs Wafer

### 6.2.1 The effects of the Surface Conditions of the GaAs Wafer on Antibacterial Activity

The effects of the surface conditions of the GaAs wafer on the Antibacterial activities are evaluated based on the presence or absence of the re-cleaning process and angle differences in the surface cut orientation.

#### 6.2.1-1 Samples

[1] Sample A: condition of a regular, shipped product (no-tilt; re-cleaned after long-term storage(note 1))

[2] Sample B: condition of a stock product (no-tilt; no re-cleaning process)

[3] Sample C: condition of a regular, shipped product with differences in the surface orientation (2° tilt-off product)

(Note 1): After products are stocked more than two months, the oxidized film on the surface will be removed by a re-cleaning process to change it back into the same condition as new products and shipped.
A blank control sample was prepared by cutting the stomacher® bag (polyethylene, PE).

The effect of the difference in film thickness of the natural oxidation layer can be analyzed by comparing the results of samples A (with a re-cleaning process) and B (without a re-cleaning process); the effect of the difference in the surface cut

orientations can be analyzed by comparing the results of samples A (no-tilt) and C (2° tilt-off).

A 4" wafer, which is a popular size these days, was purchased and then cut with a diamond pen to a size so that it fits in a 9 cm dish.

#### 6.2.1-2 Results

(1) Means and Standard Deviations of Antibacterial Activity Values

Table13 Means and Standard Deviations of Antibacterial Activity Values

| Sample | Mean | Standard deviation |
|---|---|---|
| GaAs Wafer A | 2.54 | 1.79 |
| GaAs Wafer B | 1.71 | 0.83 |
| GaAs Wafer C | 2.22 | 1.45 |

(N = 15)

The results show that the re-cleaning process increased the Antibacterial activities and also the variations.

The results also indicate that the difference in the cut angles of the surface orientations have a less significant effect on the Antibacterial activities compared with the presence or absence of the re-cleaning process.

In this test, there were differences in the experimental conditions between the laboratories, including the time passed from when the sample was removed from the protective case to the test (i.e., the difference in the condition of the surface oxidation); the degree of light illumination during the inoculation process of the bacteria culture; type of the dishes used; the washout method.

Therefore, it was considered possible that the Antibacterial activities were affected by the factors, such as the amount of light illuminated on the GaAs Wafer, the presence of dust particles on the wafer produced in the cutting process, oxidation of the wafer surface after opening the case, and the degree of contamination of the wafer surface.

### 6.2.2 Test for Antibacterial Activities of GaAs Wafer and the Effects of the Amount of Light Illuminated on the Surface on Antibacterial Activity

#### 6.2.2-1 Samples

(1) Sample D (no-tilt; condition of a product for regular shipping)

The basic specifications are shown in 6.1, except for the substrate size used being 3" so that it fits in the 9 cm dish without cutting; this is to eliminate the possible influence of chips and fine particles that were adhering onto the wafer surface on the Antibacterial activities.

A blank control sample was prepared by cutting the stomacher® bag (polyethylene, PE).

### 6.2.2-2 Testing Method

The nitrogen-filled packages were opened immediately before the testing to minimize the change of the surface condition affected by the storage environment or time passed after opening, and any other chances of contamination. Also during the testing, the amount of light that illuminated the wafer samples was cut off as much as possible. (The luminous intensity was measured whenever possible.)

Furthermore, at laboratory E, the effect of light on the Antibacterial activities of GaAs wafer was evaluated by illuminating with an intense light beam on the wafer samples in the testing.

### 6.2.2-3 Testing Results

(1) Estimation of mean, standard deviation, and expanded uncertainty (U) for the Antibacterial activity values

Table14
Mean, Standard Deviation, and Expanded Uncertainty (U) for the Antibacterial Activity Values

| Sample | Mean | Standard deviation | Expanded uncertainty (U) |
|---|---|---|---|
| No light illumination (all the laboratories) | | | |
| GaAs wafer D no light | 2.57 | 1.20 | 2.78 |
| | | | (N = 15) |
| Presence and absence of illumination (laboratory E only) | | | |
| GaAs wafer D no light | 3.70 | - | - |
| GaAs wafer D illuminated | 4.21 | - | - |
| | | | (N = 3) |

Table15 An Example of the estimation of uncertainty based on the result values obtained by the five laboratories for the testing of GaAs Wafer Sample D

Sample D

| Lab | 1 | 2 | 3 | **Total** | **SD[1]** | **Mean** | **Mean of means** |
|---|---|---|---|---|---|---|---|
| A | 1.96 | 1.50 | 1.58 | **5.04** | **1.20** | 1.68 | 2.57 |
| B | 3.20 | 2.79 | 2.91 | **8.90** | | 2.97 | |
| C | 0.79 | 0.74 | 0.86 | **2.39** | | 0.80 | |
| D | 3.93 | 3.63 | 3.48 | **11.04** | | 3.68 | |
| E | 3.80 | 3.76 | 3.57 | **11.13** | | 3.71 | |

| **Source** | **SS[2]** | **Df[3]** | **Variance** | **F statistic** | **Estimates of variance** |
|---|---|---|---|---|---|
| **Lab (A)** | 19.88 | 4 | 4.97 | 141.12 | (σe)2+3(σa)2 |
| **Error (e)** | 0.35 | 10 | 0.04 | 0.02 | (σe)2 |
| **Total** | 20.23 | 14 | 1.44 | | |
| | | | | F (0.05, 4, 10)=3.48 | |
| σe | 0.19 | | | Statistically significant | |
| σa | 1.28 | 1.644712 | | t (0.05, 14)=2.145 | |
| uc | 1.30 | 1.679926 | | | |
| U | 2.78 | | | Antimicrobial activity=2.57±2.78 | |

Note: [1] SD = standard deviation; [2] SS = sum of squares; [3] Df = degrees of freedom

The results for laboratory E show that the Antibacterial activities of GaAs wafers are influenced by the degree of light illumination. However, it is not clear to what degree the microbial activity of GaAs wafers are affected by light under the regular testing conditions, since the luminous intensity in this test was considerably high.

The results of two tests show that the variation between the laboratories is greater than that within each laboratory, and it was mentioned in the microbial activity test for the GaAs wafer that some factor which has not been considered previously might have contributed to the variation.

### 6.2.3 Antibacterial Activity Test for GaAs Wafers

A reproducibility test under the no-light condition, which is described in 6.2.2, was carried out only by laboratory E.

#### 6.2.3-1 Samples

(1) GaAs wafer

Sample E (no-tilt; re-cleaning)

Five wafers with the same specifications as sample A (no-tilt; re-cleaning) were used. The 4" wafers were used without cutting and placed in a square dish for evaluation. A blank control sample was prepared by cutting the stomacher® bag (polyethylene, PE).

#### 6.2.3-2 Testing Method

The testing was performed using the same method as described in 6.2.2, with the same conditions, including the light condition.

#### 6.2.3-3 Testing Results

(1) Mean and Standard Deviation for the Antibacterial Activity Values

Table16 Mean and Standard Deviation for the Antibacterial Activity Values

| Sample | Mean | Standard deviation |
|---|---|---|
| GaAs wafer E<br>no light | 4.61 | 0.52 |

(N = 5)

The result indicates that the reproducibility for the Antibacterial activity of GaAs wafer under the no-light condition was not observed.

## 6.3 Eluate Composition and Antibacterial Activities of GaAs Wafer

### 6.3.1 Purpose

The purpose is to evaluate the Antibacterial activity of the GaAs wafer in terms of the composition of the eluates of gallium and arsenic components.

### 6.3.2 Materials

The SCDLP (soybean-casein digest broth with lecithin polysorbate) medium that was used for the washing process in 5.2.1 was used as a test material. The material was sampled from laboratories B and D, which yielded the lower and higher values of Antibacterial activity, respectively.

### 6.3.3 Method of Evaluation

Evaluation is carried out based on the relation of the amount of Ga and As, and the viable counts (log) in the SCDLP broth medium that was used in the washing process of the Antibacterial activity testing. The eluate composition for the gallium was measured with inductively coupled plasma optical emission spectroscopy (ICP-OES), while that for the arsenic was measured with atomic absorption spectrophotometry (AAS).

#### 6.3.3-1 Measurement of Arsenic

(1) Preparation of the testing solution

0.1 to 0.2 gram of the sample was measured in a Kjeldahl flask and wet-digested with nitric acid, sulfuric acid, and perchloric acid. Then a proper amount of water and saturated ammonium oxalate were added and re-heated until white fumes appeared. After cooling, all of the solution was filtered into a 50 ml flask using filter paper (No. 5C [Toyo Roshi Kaisha, Ltd.]), and 5 ml of 40% potassium iodide solution was added and set aside. After 30 minutes, 5 ml of 10% ascorbic acid solution was added, and 50 ml of the test solution was made by adding a proper amount of water.

(2) Preparation of standard solution

From the standard arsenic solution (0.1 ppm (prepared before use)), 1, 2, 4, 6, 8, 10, 12 ml amounts were measured into volumetric flasks (100 ml), and a proper amount of water and 10 ml of sulfuric acid were added, and then 10 ml of 40% potassium iodide solution was added and set aside in the same way as for the testing solution. After 30 minutes, 10 ml of 10% ascorbic acid solution was added, and 100 ml of solution was made by adding a proper amount of water.

(3) Measurement

0.6% sodium-borohydride-0.5% sodium-hydroxide solution and hydrochloric acid (5+1) was set, and the standard solution and testing solution were inserted in the hydride generation atomic absorption spectrophotometry and measurement was performed.

(4) Condition for the operation of atomic absorption spectrophotometry

Model: SpectrAA 220 [Varian Technologies Japan Limited]
Hydride generator: VGA-77 [Varian Technologies Japan Limited]
Gas pressure: Argon 0.4 kgf/cm$^2$
Wavelength measured: 193.7 nm
Quartz heating cell temperature: 925°C

### 6.3.3-2 Measurement of Gallium

(1) Preparation of Testing Solution

A 5 ml of nitric acid was added to 0.5 g to 1.0 g of sample, and was evaporated to dryness. Then 500 µl of nitric acid and water were added and heated for 30 minutes, adding water to make 50 ml of testing solution.

(2) Preparation of Standard Solution

The standard solution was prepared by adding 500 µl of nitric acid to 50 µl of commercially available gallium standard solution (1000 µg/ml), and water was added to make 50 ml of stock solution. The stock solution was diluted with 1%-nitric acid solution, and prepared to 0, 0.01, 0.05, and 0.1 µg/ml standard solutions.

(3) Measurement

The standard solution and the testing solution were placed in the ICP-OES and measured.

(4) Operational condition of ICP optical emission spectrometer

Model: Optima 3300DV [PerkinElmer Japan Co., Ltd.]
High frequency output: 1,300 W
Gas flow: argon (plasma gas) 15 L/min
argon (auxiliary gas) 0.5 L/min
argon (carrier gas) 0.80 L/min
Sample uptake rate: 1.00 mL/min
Nebulizer: cross flow type
Plasma viewing: axial
Wavelength measured: 294.364 nm (gallium)

### 6.3.4 Testing Results

Table17 Elution Levels of Gallium and Arsenic from GaAs and Viable Counts (Log)

| | Laboratory B | | | Laboratory D | | |
|---|---|---|---|---|---|---|
| | Ga (ppm) | As (ppm) | Viable count (log) | Ga (ppm) | As (ppm) | Viable count (log) |
| GaAs wafer A | 3.6 | 4.0 | 5.12 | 1.0 | 1.7 | 3.92 |
| GaAs wafer B | 3.0 | 3.4 | 4.98 | 1.6 | 1.7 | 4.19 |
| GaAs wafer C | 3.3 | 3.6 | 5.16 | 1.4 | 2.0 | 3.91 |
| | | | | | | |
| Blank film | <1 | <0.1 | 6.43 | <1 | <0.1 | 5.92 |

(Mean values for N = 3)

No correlation was observed between the levels of elution for gallium and arsenic from the GaAs wafer and the viable counts (log).

## 7. Summary of the Investigative Research for Three Years

In fiscal 2001, the first year of the investigative research, factors that could affect the uncertainty measurement in the Antibacterial activity testing were thoroughly examined (drawing a cause and effect diagram). Three factors were chosen: strain for testing; type of medium; and culture containers. Requirements for standard testing samples, which would be used to estimate uncertainty, were reviewed and the samples were made experimentally.

The concentration levels of Antibacterial agent and the stability of Antibacterial effectiveness were examined for the silver acrylic coated film that was made as a trial sample. Although there are problems, it was suggested that silver acrylic coated film is a useful material as a standard testing material.

In the literature review, the scheme of certification of standard reference material for the measurement of enzyme activity by the Japan Society of Clinical Chemistry (JSCC) and the relation to the clinical measurement practice was investigated. It was decided to examine if such a scheme could be built in the area of Antibacterial activity for the purpose of establishment of traceability.

In fiscal 2002, silver acrylic coated film, which was regarded as a promising secondary standard testing material was continuously examined for completion. At the same time, a potential primary standard testing material, which is supposed to have higher precision and accuracy, was experimentally made and selected.

Two primary standard testing material candidates were selected: (1) quartz glass material to which silver ion was added by ion implantation, (2) GaAs wafer, which is used as a semiconductor substrate. For material (1), trial samples were made; for material (2), commercially available products were obtained, and the Antibacterial activity testing was performed for evaluation.

With respect to (1) Ag+ ion implanted glass, it was determined that the use of this material as a standard testing material was difficult due to the technical problems with the ion implantation method and the production cost. On the other hand, regarding the material (2) GaAs wafer, it was found that the material is not repeatedly usable due to the elution of gallium and arsenic, and the first test yielded Antibacterial activity values ranging from 2 to 3. Although there is a condition of one time use only, this material has a clear physicochemical specification and is mass-produced as an electronic part, so that they are readily obtainable; therefore, it was suggested that the material is favorable as the primary standard testing material. Accordingly, it was decided to examine the possibilities and the number of laboratories to add for the further testing in the next fiscal year.

With respect to the evaluation of silver acrylic coated film, in the first test, it was observed that the bacterial suspension on the sample swelled and expanded larger than the original size (4 cm × 4 cm) due to the water solubility of the coating properties. It was determined to partially change the testing method and use a method in which the sample and the cover film are kept in an inverted position.

The homogeneity of the amount of silver in the coating agent was ensured by measuring the amount of silver. However, it was suspected that the Antibacterial activity values were affected by the change in the amount of residual ammonium in the coating, depending on the drying condition of the coated film. Therefore, it became clear that it is important to be able to strictly control the heating and drying processes in order to produce standard

testing samples with stable Antibacterial activity values; and as one of those control items, it is also important to specify the amount of the residual ammonium as a specification.

In the literature review, the testing conditions (i.e., strains, culture temperature, culture time) defined in the methods used in Europe and the United States for the Antibacterial and bacteriostasis testing utilizing bacteria were studied. The literature regarding the estimation of uncertainty in the testing for the area of microbiology was also reviewed.

In fiscal 2003, reproducibility testing was carried out by the five laboratories for the estimation of uncertainty in the Antibacterial activity testing using two types of samples, GaAs wafer and silver acrylic coated film, which were selected as the candidates for standard testing materials.

Uncertainty was estimated based on the results, and it is considered that these standard testing materials can also contribute to the establishment of traceability, serving as consensus standard materials.

The method of estimation of uncertainty described in this report provides an example of uncertainty estimation in the Antibacterial activity testing, which could not have been possible without the homogeneous and stable standard testing materials.