The Statistical Analyzed Report of the Round Robin Test

(Antibacterial/ISO 20743)

20th November, 2019

APEC Project SCSC 01 2018T

Capacity Building on Testing Methods for Functionality Finishing on Textile Products and Certification Methods within the APEC Region
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1. Foreword
   This round robin tests have been conducted according to ISO/IEC 17025:2017 "General requirements for the competence of testing and calibrations laboratories", and ISO/IEC 17043:2010 "Conformity Assessment-General requirements for proficiency testing"

2. Purpose of the Round Robin Test
   APEC project SCSC 01 2018T-Capacity Building on Testing Methods for Functionality Finishing on Textile Products and Certification Methods within the APEC Region has been conducted according to the APEC Project Proposal from August 2018 to December 2019.
   In this project, following 4 functionality finishing testing method standards (ISO 20743, ISO 17299-1 to 5, ISO 13629-1, ISO 18184) have been explained.
   And, these round robin tests were carried out to confirm whether the participants obtained sufficient skill and knowledge of those ISO standards to harmonize the testing methods in the APEC region, in 3 seminars, 1st seminar in Washington DC, 2nd seminar in Jakarta, and 3rd seminar in Taipei city.

3. Testing Standards
   Round robin tests were carried out on the following 2 standards within above 4 standards.

   > ISO 20743: Textile-Determination of antibacterial activity of textile products”
     Test method: B.1.1 Absorption method
     Quantitative measurement: plate count method
     Test strain: Staphylococcus aureus (WDCM code 00193)

     Test method: Part2: Detector tube method
     Test odour: Ammonia (NH3)

4. Executing Agency
   This round robin tests have been carried out by Japan Textile Evaluation Technology council (JTETC) and its members.
   Test specimens were made by KURABO Industries ltd.
   Test results of antibacterial test were statistical analyzed by KE’KEN Textile Testing & Certification Center.
   And, test results of deodorant test were statistical analyzed by BOKEN Quality Evaluation Institute.
5. Testing Program Scheme
This round robin tests have been carried out according to the scheme of proficiency test in ISO/IEC 17043:2010.

6. Participating Laboratory
28 laboratories from 8 APEC members participated in this round robin test for antibacterial test.

But, 3 laboratories from 3 APEC members did not submit their data sheets of the test results in dead line of the submitting.

Therefore, statistical analysis was calculated excluding those data.
And, the passwords applied by laboratories are listed instead of the names of laboratories in this report.

7. Method of Statistical Analysis and Evaluation
Statistical analysis was performed according to ISO/IEC 17043:2010 and ISO 13528 “Statistical methods for use in proficiency testing by interlaboratory comparisons”.

So, as robust statistical technique, Z score is calculated by median and normalized interquartile range (NIQR) to evaluate testing result as following.

\[
|Z| \leq 2 : \text{Satisfactory} \\
2 < |Z| < 3 : \text{Questionable} \\
|Z| \geq 3 : \text{Unsatisfactory}
\]

8. Testing Specimen
(1) Testing Specimen for antibacterial finished (black color)

Size: 30cm/30cm
Material: polyester 65%/cotton 35%
Yarn Count: 45/45
Fabric Density: 136/inch / 72/inch
Processing conditions and equipment: following table
Table: Processing conditions and equipment of testing Specimen

<table>
<thead>
<tr>
<th>Process</th>
<th>Equipment</th>
<th>Processing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spinning</td>
<td>Ring spinning frame</td>
<td></td>
</tr>
<tr>
<td>Weaving</td>
<td>Air-jet loom</td>
<td></td>
</tr>
<tr>
<td>Preparation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singeing</td>
<td>Continuous range</td>
<td>The flame of the gas burner</td>
</tr>
<tr>
<td>Desizing</td>
<td></td>
<td>Enzyme and oxidative desizing</td>
</tr>
<tr>
<td>Scouring and</td>
<td></td>
<td>Steam treatment : 98°C × 30 min</td>
</tr>
<tr>
<td>Bleaching</td>
<td>Mercerizing machine</td>
<td>35%(\text{H}_2\text{O}_2) / NaOH aq.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Steam treatment : 98°C × 30 min</td>
</tr>
<tr>
<td></td>
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<td>NaOH aq.</td>
</tr>
<tr>
<td>Dyeing</td>
<td>Continuous</td>
<td>Dipping – Dry – Fix – wash - Dry</td>
</tr>
<tr>
<td></td>
<td>dyeing machine</td>
<td></td>
</tr>
<tr>
<td>Finishing</td>
<td>Pad-dry-cure method</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tenter machine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Antibacterial agent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Quaternary ammonium salt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Deodorizing agent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Metal salt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drying : 125°C × 2 min</td>
</tr>
</tbody>
</table>

(2) Control Specimen (white color)
100% cotton fabric defined in clause 3.1 of ISO 20743: 2013

9. Schedule
>Deadline for application: 24 July, 2019
>Delivery of Test Specimens: In August, 2019
>Deadline for submission of Test Result: 30 September, 2019
>Publication of Test Results Report: In November, 2019
>Study of Test Results: 20-21 November, 2019 In the 4th seminar in Shanghai

10. Test Results and Statistical Analysis
10.1 Test results reported by participating laboratories
Annex A-1 shows test results reported from participating laboratories. Variations in the number of digits in data are the results of faithfully transcribing figures submitted by each of the participating laboratories. Of 25 laboratories, 24 laboratories met the test condition requirements stipulated in ISO20743, validating their tests. The test at the remaining laboratory was not validated, but its results were transcribed as submitted.
10.2 Summary of test results

The cells in the z-score judging column containing "$\circ$" indicate "unsatisfactory," in which the absolute value of the z-score is no less than 3. The cells containing "!" indicate "questionable" with the absolute value of the z-score greater than 2 and less than 3. NBRC12732 was specified as the test strain, and laboratories that only had other strains used a strain they had available. Three laboratories used ATCC6538 and two laboratories used ATCC6538P.

Annex A-2 shows the histogram of test results, and Annex A-3 shows the bar chart for z-scores.

10.3 Evaluation of statistical analysis results

The test results (antibacterial activity values) of all participating laboratories (N=25) were evaluated, based on the z-score, to find that none of the laboratories had the z-score for its antibacterial activity value indicating "unsatisfactory". Three laboratories returned "questionable" evaluation.

The smallest z-score was -2.97 (antibacterial activity value of 4.6) and the largest z-score was 2.97 (antibacterial activity value of 6.8). The median antibacterial activity value for all participating laboratories was 5.70, with the normalized interquartile range of 0.37.

11. Summary of the Results and Technical considerations.

11.1 Results and adequacy of test specimens' homogeneity confirmation test

Annex B shows the results of homogeneity confirmation test on test specimens used in the round-robin test.

The overview of the homogeneity confirmation test, conducted this time, is as follows: The center part of processed cloth prepared was divided into 100 equal portions. Ten pieces were randomly chosen and sent to a pre-selected laboratory for testing. Since ISO 20743 stipulates the use of the mean of antibacterial activity values taken from three locations, the laboratory was asked to supply repeating data from three locations for analysis.

ISO 13528 confirms sufficient homogeneity

if: Standard deviation between specimens $s_s \leq 0.3 \times \text{Standard deviation of proficiency test: } \sigma$

In this study, $s_s = 0.10$ and the results of the round-robin test was $\sigma = 0.45$, indicating that the standard deviation between specimens was smaller than 0.3 times the standard deviation of proficiency test (0.135).

It was therefore confirmed after the round-robin test that the test specimens were homogeneous.
11.2 Summary and considerations of the proficiency test

① Laboratory No.8, which has a low antibacterial activity value, and Laboratories No.23 and No.25, which have a high antibacterial activity value, were rated as “questionable.”

② Stratified analysis of data excluding ATCC bacteria results (reference values) put Laboratories No.23 and No.25, which were rated “questionable” in all-test data, in the “unsatisfactory” rating, while adding Laboratories No.3, No.18 and No.31, which had a relatively low antibacterial activity value, to the “questionable” rating.

③ The histogram for test results (all 25 laboratory data) was the highest: at 5.5 and 6.0, which are near the median value (5.70), and declined almost symmetrically to both sides. It was therefore decided that the data was in a state close to normal distribution, and put to statistical analysis.

12. Conclusion

Although there were some deviations in the test method, but the test results of this round robin test were generally good.

And, the purpose of this round robin test, which is confirmation whether the participants obtained sufficient skill and knowledge of ISO standards in the seminars to harmonize the testing methods in the APEC region, has been achieved.
## Annex A-1  Test Results, Statistical Analysis and z-score

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Password</th>
<th>Concentration of nocardia (CFU/ml)</th>
<th>Control specimen (Standard cloth)</th>
<th>Test specimen (Antibacterial finished cloth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>immediately after incubation after incubation</td>
<td>growth value</td>
<td>immediately after incubation after incubation</td>
</tr>
<tr>
<td>logC0</td>
<td>logCt</td>
<td>F</td>
<td>logT0</td>
<td>logTf</td>
</tr>
<tr>
<td>1</td>
<td>22670321</td>
<td>1.6 x 10⁵</td>
<td>4.48</td>
<td>6.97</td>
</tr>
<tr>
<td>2</td>
<td>BR06406</td>
<td>1.01 x 10⁵</td>
<td>4.55</td>
<td>6.68</td>
</tr>
<tr>
<td>3</td>
<td>Bilisbu2</td>
<td>1.7 x 10⁵</td>
<td>4.4</td>
<td>6.9</td>
</tr>
<tr>
<td>4</td>
<td>19800914</td>
<td>1.2 x 10⁵</td>
<td>4.30</td>
<td>6.71</td>
</tr>
<tr>
<td>5</td>
<td>6s653157</td>
<td>1.3 x 10⁵</td>
<td>4.53</td>
<td>7.18</td>
</tr>
<tr>
<td>6</td>
<td>00090105</td>
<td>1.7 x 10⁵</td>
<td>4.2</td>
<td>6.6</td>
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<tr>
<td>7</td>
<td>3t0k1b9k</td>
<td>1.1 x 10⁵</td>
<td>4.48</td>
<td>7.20</td>
</tr>
<tr>
<td>8</td>
<td>20190619</td>
<td>1.4 x 10⁵</td>
<td>4.4</td>
<td>5.9</td>
</tr>
<tr>
<td>9</td>
<td>8tahue8</td>
<td>1.3 x 10⁵</td>
<td>4.48</td>
<td>7.18</td>
</tr>
<tr>
<td>10</td>
<td>neccbio</td>
<td>2.09 x 10⁵</td>
<td>4.56</td>
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<td>6.93</td>
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<td>1.2 x 10⁵</td>
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<td>6.85</td>
</tr>
<tr>
<td>14</td>
<td>62002179</td>
<td>1.2 x 10⁵</td>
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<td>7.08</td>
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<tr>
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<td>JDIF2019</td>
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<td>4.5382</td>
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<td>1.89 x 10⁵</td>
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<td>7.24</td>
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<td>4.6</td>
<td>7.1</td>
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<tr>
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<td>Shiken01</td>
<td>1.1 x 10⁵</td>
<td>4.2</td>
<td>6.9</td>
</tr>
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<td>5.2 x 10⁵</td>
<td>4.9</td>
<td>7.1</td>
</tr>
<tr>
<td>22</td>
<td>7F58933</td>
<td>2.3 x 10⁵</td>
<td>4.54</td>
<td>7.08</td>
</tr>
<tr>
<td>23</td>
<td>DAIWA48H</td>
<td>1.35 x 10⁵</td>
<td>4.31</td>
<td>7.85</td>
</tr>
<tr>
<td>24</td>
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<td>2.1 x 10⁵</td>
<td>4.4</td>
<td>7.3</td>
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<tr>
<td>25</td>
<td>15OCT539</td>
<td>2.91 x 10⁵</td>
<td>4.77</td>
<td>6.55</td>
</tr>
</tbody>
</table>

| number of data | 25 | 25 | 25 | 25 | 25 | 25 |
| median value   | 4.49 | 7.04 | 2.50 | 4.40 | 1.30 | 5.70 |
| first quartile | 4.40 | 6.85 | 2.40 | 4.28 | 1.30 | 5.40 |
| third quartile | 4.55 | 7.18 | 2.70 | 4.50 | 1.30 | 5.50 |
| interquartile range | 0.15 | 0.33 | 0.30 | 0.22 | 0.00 | 0.50 |
| normalized interquartile range | 0.11 | 0.24 | 0.22 | 0.17 | 0.00 | 0.57 |
| robust coefficient of variation (%) | 2.5 | 3.5 | 8.9 | 3.8 | 0.0 | 65 |
| minimum value  | 4.20 | 5.90 | 1.50 | 1.30 | 0.00 | 4.60 |
| maximum value  | 4.90 | 7.85 | 3.50 | 4.76 | 1.30 | 6.60 |
| range          | 0.70 | 1.95 | 2.00 | 3.46 | 1.30 | 2.20 |

NOTE The results were transcribed as submitted.

Average value (reference) 5.70
Standard deviation (reference) 0.45
Annex A-1 Test Results, Statistical Analysis and z-score

(1) Test Data(excluding ATCC 6538 and ATCC 6538P) [reference]

<table>
<thead>
<tr>
<th>Lab No.</th>
<th>Password</th>
<th>Concentration of inoculum (CFU/mL)</th>
<th>Control specimens (Standard cloth)</th>
<th>Test specimens (Antibacterial finished cloth)</th>
<th>z-score</th>
<th>Judgment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>immediately after inoculation</td>
<td>Number of bacteria (Logarithmic value)</td>
<td>Number of bacteria (Logarithmic value)</td>
<td>Growth value</td>
<td>immediately after inoculation</td>
</tr>
<tr>
<td>3</td>
<td>Biselbu2</td>
<td>$1.7 \times 10^5$</td>
<td>4.4</td>
<td>6.9</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
<td>4</td>
<td>1960914</td>
<td>$1.2 \times 10^5$</td>
<td>4.30</td>
<td>6.71</td>
<td>2.4</td>
<td>4.30</td>
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<td>7.18</td>
<td>2.7</td>
<td>4.57</td>
</tr>
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<td>7</td>
<td>3tky1b9k</td>
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<td>4.48</td>
<td>7.20</td>
<td>2.7</td>
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<td>6.85</td>
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<td>16</td>
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<td>7.1771</td>
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<td>7.1</td>
<td>2.5</td>
<td>4.1</td>
</tr>
<tr>
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<td>6.9</td>
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<td>4.2</td>
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<td>3.98</td>
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<td>4.77</td>
<td>6.55</td>
<td>1.78</td>
<td>4.76</td>
</tr>
</tbody>
</table>

number of data: 20  
median value: 4.52  
first quartile: 4.42  
third quartile: 4.57  
interquartile range: 0.14  
normalized interquartile range: 0.11  
robust coefficient of variation (%): 2.3  
minimum value: 4.20  
maximum value: 4.90  
range: 0.70

**Average value (reference):** 5.77  
**Standard deviation (reference):** 0.39

NOTE The results were transcribed as submitted.
Annex A-2  The histogram of test results

Test data (All)

<table>
<thead>
<tr>
<th>Antibacterial Activity Value</th>
<th>Number of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4.5</td>
<td>2</td>
</tr>
<tr>
<td>5.0</td>
<td>2</td>
</tr>
<tr>
<td>5.5</td>
<td>8</td>
</tr>
<tr>
<td>6.0</td>
<td>12</td>
</tr>
<tr>
<td>6.5</td>
<td>2</td>
</tr>
<tr>
<td>7.0</td>
<td>2</td>
</tr>
</tbody>
</table>

Test data (excluding ATCC 6538 and ATCC 6538P)

<table>
<thead>
<tr>
<th>Antibacterial Activity Value</th>
<th>Number of Laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4.5</td>
<td>2</td>
</tr>
<tr>
<td>5.0</td>
<td>2</td>
</tr>
<tr>
<td>5.5</td>
<td>8</td>
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<td>6.0</td>
<td>12</td>
</tr>
<tr>
<td>6.5</td>
<td>2</td>
</tr>
<tr>
<td>7.0</td>
<td>2</td>
</tr>
</tbody>
</table>
Annex A-3 The bar chart for z-scores

z-score (All data)

z-score (excluding ATCC 6538 and ATCC 6538P)

Lab No.
Annex B

Homogeneity evaluation results for test specimens

1. Test data

- An antibacterial test (ISO20743, 8.1 Absorption method, Plate count method) was carried out on ten specimens, chosen from test specimens, at the Laboratory 1. [Table 1] shows the results. [Figure 1] shows a graph for examining data dispersion.

[Table 1]

<table>
<thead>
<tr>
<th>Specimen No</th>
<th>N1</th>
<th>N2</th>
<th>N3</th>
<th>Xt (Mean of specimens)</th>
<th>Wt (Range among test areas)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>0.0</td>
</tr>
<tr>
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<td>75</td>
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<td>0.0</td>
</tr>
</tbody>
</table>

Sx (Standard deviation of mean of specimens) = 0.10
Sw (Standard deviation within specimens) =$\sqrt{\frac{\sum Wt^2}{30}}$ = 0.05
Ss (Standard deviation between specimens) =$\sqrt{\frac{(Sx^2-Sw^2)}{3}}$ = 0.10

$t = 1, 2, \ldots \ldots 10$

Ss = 0.10 ≤ 0.3 × $\sigma$ = 0.3 × 0.45 = 0.135
($\sigma = 0.45$ is the standard deviation described in Annex A-1 (1)).

The antibacterial activity value for N1–N3 was calculated as $(\log C_i$ of the mean) – $(\log I_i$ for N1–N3).

[Figure 1]
2. Confirmation of the homogeneity of spent test specimens based on ISO 13528 rules. 
[Table 2] shows the ISO 13528 rules.

<table>
<thead>
<tr>
<th>Rules shown in ISO 13528</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_s \leq 0.3 \times \sigma \Rightarrow$ Homogenous</td>
</tr>
</tbody>
</table>

$S_s$ : Standard deviation between specimens
$\sigma$ : Standard deviation of proficiency testing

As shown in [Table 1], the standard deviation between specimens ($S_s = 0.10$) is smaller than the standard deviation of proficiency testing ($\sigma = 0.45$), confirming that the test specimens are homogenous.

END
Annex C

APEC PROJECT SCSC 01 2018T

Notification for Round Robin Tests

Thank you for your participation for Round Robin Tests.
Please note the following before carry out the tests.

1. Following has been sent from us.
   ■ This Notification
   ■ Procedures for Round Robin Test (for antibacterial test / for deodorant test)
   ■ Data Sheets (for antibacterial test / for deodorant test)
   □ Testing specimens (antibacterial finished (Black) / deodorant finished (dark blue))
   □ Control specimen (White) (only for antibacterial test)
   * You can download “■” items documents from the following website of JTETC.

2. Testing Standard
   (A) ISO 20743:2013 _Textile-Determination of antibacterial activity of textile products_
      Test method: 8.1 Absorption method
      Quantitative measurement: Annex C; plate count method
      Testing strain: Staphylococcus aureus (WDCM code 00193)
   (B) ISO 17299-1-5:2014 _Textile Determination of deodorant property_
      Test method: Part2; Detector tube method
      Testing odour: 7.1.1 Ammonia (NH₃)
      □ If you take another method form above one, please note that on the Data Sheets.

3. Submission of Test Results (Data Sheets)
   (A) How to submit
      By e-mail to following address
      To) Mr. N. Suso   suso@sengikyo.or.jp
   (B) Deadline for submission
      30 September, 2019
   (C) Others
      If you have any question, please contact following person.
      To) Ms. S Nishikawa (antibacterial test)  s-nishikawa@jwif.org
      To) Mr. K. Kawabata (deodorant test)  k-kawabata@boken.or.jp
      CC) Mr. N. Suso   suso@sengikyo.or.jp

4. Report Statistically analyzed of Round Robin Test data
   You can see this report on the following website of JTETC in November, 2019.
Annex D

Data sheet of round robin test for antibacterial (ISO 20743)

Reporting date:

<table>
<thead>
<tr>
<th>APEC Member Economy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory (Organization)</td>
<td></td>
</tr>
<tr>
<td>Participant (Operator name)</td>
<td></td>
</tr>
<tr>
<td>Your Password</td>
<td></td>
</tr>
<tr>
<td>Test date (inoculation date)</td>
<td></td>
</tr>
</tbody>
</table>

1. Test using cloth specimens
1.1 The bacteria concentration of test inoculum

<table>
<thead>
<tr>
<th>Number of colonies</th>
<th>Dilution rate</th>
<th>Concentration of inoculum (CFU/mL)</th>
<th>Strain number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petri dish 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petri dish 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.2 Measurement of bacterial count immediately after inoculation
1.2.1 Control specimens (Standard cloth)

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Numbers of colonies</th>
<th>Dilution rates</th>
<th>Shake-out physiological saline (ml)</th>
<th>Numbers of bacteria ( C_0 ) (Average number)</th>
<th>( \log C_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petri dishes 1</td>
<td>Petri dishes 2</td>
<td>Average numbers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.2.2 Test specimens (Antibacterial finished cloth)

<table>
<thead>
<tr>
<th>Specimens</th>
<th>Numbers of colonies</th>
<th>Dilution rates</th>
<th>Shake-out physiological saline (ml)</th>
<th>Numbers of bacteria ( T_0 ) (Average number)</th>
<th>( \log T_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petri dishes 1</td>
<td>Petri dishes 2</td>
<td>Average numbers</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.3 Measurement of bacterial count after incubation
1.3.1 Control specimens (Standard cloth)

| Specimens | Numbers of colonies | Dilution rates | Shake-out physiological saline (ml) | Numbers of bacteria | Number of bacteria $G$ (Average number) | log $G$
|------------|---------------------|----------------|----------------------------------|---------------------|----------------------------------------|-------
| 1          |                     |                |                                  |                     |                                        |       
| 2          |                     |                |                                  |                     |                                        |       
| 3          |                     |                |                                  |                     |                                        |       

1.3.2 Test specimens (Antibacterial finished cloth)

| Specimens | Numbers of colonies | Dilution rates | Shake-out physiological saline (ml) | Numbers of bacteria | Number of bacteria $T_1$ (Average number) | log $T$
|------------|---------------------|----------------|----------------------------------|---------------------|----------------------------------------|-------
| 1          |                     |                |                                  |                     |                                        |       
| 2          |                     |                |                                  |                     |                                        |       
| 3          |                     |                |                                  |                     |                                        |       

1.4 Test result

<table>
<thead>
<tr>
<th>Judgement of test effectiveness</th>
<th>Control specimens</th>
<th>Test specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Difference of extremes for numbers of bacteria (common logarithm) of three specimens $\leq 1$</td>
<td>Difference of extremes for numbers of bacteria (common logarithm) of three specimens $\leq 2$</td>
</tr>
<tr>
<td></td>
<td>Immediately after inoculation</td>
<td>Immediately after inoculation</td>
</tr>
<tr>
<td></td>
<td>After inoculation</td>
<td>After inoculation</td>
</tr>
<tr>
<td></td>
<td>Difference (Judgement : )</td>
<td>Difference (Judgement : )</td>
</tr>
<tr>
<td></td>
<td>$= $</td>
<td>$= $</td>
</tr>
<tr>
<td></td>
<td>$= $</td>
<td>$= $</td>
</tr>
<tr>
<td></td>
<td>$= $</td>
<td>$= $</td>
</tr>
</tbody>
</table>

Antibacterial activity value : $A = \log C_1 - \log C_0 - \log T_1 - \log T_0$

$A =$
Annex E

APEC PROJECT SCSC 01 2018T
Procedures for Round robin test ~antibacterial activity~

Table of contents

- Methods covered in this round robin test
- Test procedure
- Specified matters in the round robin test
- Test report

Table of contents

- Methods covered in this round robin test
- Test procedure
- Specified matters in the round robin test
- Test report

ISO 20743
Textiles - Determination of antibacterial activity of textile products

• Absorption method
an evaluation method in which the test bacterial suspension is inoculated directly onto samples specimens
**Quantitative measurement**

**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.

**Luminescence method**
- Method in which the amount of ATP contained in bacterial cells is measured.

**Apparatus required to carry out this test**

- **Spectrophotometer**, capable of measuring at a 620nm to 660nm wavelength (or McFarland’s nephelometer)
- **Incubator**, capable of maintaining a constant temperature of 37°C ± 2°C
- **Clean bench (or Safety cabinet)**, for microbial test
- **Balance**, which can be read to the nearest 0.01g
- **Pipette**, having the most suitable volume for each use, with a tip made of glass or plastic

**Apparatus required to carry out this test**

- **Vials**, 30ml glass bottles, with screw openings, polytetrafluoroethylene or silicone packing and caps made of suitable material.
- **Petri dishes**, that have been sterilized, made of glass or plastic, in diameter sizes of 90mm to 100mm
- **Erlenmeyer flask (with cap)**, of capacity 100ml
- **Metal wire basket**, for autoclaving, to be used for sterilizing test specimens
- **Aluminium foil**
- **Reciprocal incubation shaker**
- **Autoclave**, capable of sterilizing at 120°C ± 2°C and 103kPa ± 5kPa

**Table of contents**

- Methods covered in this round robin test
- Test procedure
- Specified matters in the round robin test
- Test report
**Test procedure - Absorption method -**

**Incubation**

1. Pick up the preserved stock bacteria from the storage container. Streak on to the plate of Enumeration agar (EA) and incubate at 37 °C±2°C for 24h to 48h.

   **Enumeration agar (EA)**
   - Dehydrated yeast extract 2.5g
   - Casein tryptone 5.0g
   - Glucose 1.0g
   - Agar 12g to 18g (depending on the gel strength of the product)
   - Water 1000ml
   - Mix well and adjust pH, 7.2±0.2
   - Then sterilize by autoclave.

   Incubate under the following conditions:
   - Temperature : 37°C±2°C
   - Rate of shaking : 110 times per minute and 3cm width by reciprocal incubation shaker
   - Incubation time : 18h to 24h

2. Pour 20ml of Nutrient broth (NB) (or Tryptone soya broth (TSB)) into a 100ml Erlenmeyer flask. Apply an inoculating loop to pick one colony up from the incubation as specified in 1. and inoculate it in the broth.

   **Nutrient broth (NB)**
   - Beef extract 3g
   - Peptone 5g
   - Water 1000ml
   - Mix well and adjust pH, 6.9±0.2
   - Then sterilize by autoclave.

3. Pour 20ml of NB (or TSB) into a 100ml Erlenmeyer flask.

   Add 0.4ml of the inoculum from the incubation as specified in 2. that contains 1x10⁶CFU/ml to 3x10⁶CFU/ml in bacteria concentration to the flask.

   **CFU : Colony forming unit**
Test procedure - Absorption method

Preparation of test inoculum

Adjust the bacteria to a concentration of $1 \times 10^5$ CFU/ml to $3 \times 10^5$ CFU/ml by a spectrophotometer using NB (or TSB) after it has been diluted 20 times with water at room temperature.

The Prepared inoculum is preserved by ice-cooling and used within 4h.

Test procedure - Absorption method -

Preparation of test specimens

Obtain 6 test specimens and 6 control specimens with a mass of 0.40g. These are sterilized by autoclaving.

Cut the specimens into approx. 2cm squares and place them on top of one another at the bottom of the vial.

Test procedure - Absorption method -

Sterilization

1. Wrap the opening of vials containing specimens with aluminum foil.
2. Place the wrapped vials in a wire basket for autoclaving.
3. Wrap the vial caps with aluminum foil and place them in the wire basket.
4. Sterilize the caps and the vials containing the specimens by autoclave.

Process them for 15 to 20 minutes at 121°C in the autoclave, and take them out when it cools to 80 - 100°C.
Test procedure - Absorption method -

Sterilization

5. Remove the aluminum foil and allow the specimens in the vials to dry for 60 min or more by placing them on a clean bench.

6. Cap the vials.

Test procedure - Absorption method -
Test operation
Inoculation of test specimens

Inoculate each specimen with 0.2ml of the test inoculum, distributing it in several spots.

Test procedure - Absorption method -
Test operation
Shake-out after inoculation

Immediately after the inoculation, add 20ml of the shake-out physiology saline (or SCMLP medium or the neutralizing solution) into each of 3 vials in which a test specimen and each of 3 vials in which a control specimen have been placed, cap the vials and shake out. Carry out these steps swiftly.
**Shake-out physiological saline**

Mix well the following compositions, then sterilize by autoclave.

- Sodium chloride (NaCl) 8.5g
- Polysorbate 80 2.0g
- Water 1000ml

**Test procedure - Absorption method - Test operation**

**Incubation**

Incubate the 3 vials for control specimens and the 3 vials for test specimens which have not been subjected to the shake out at 37°C for 18h.

**Test procedure - Absorption method - Test operation**

Shake-out after incubation

**Quantitative measurement - Plate count method**

Take 1ml of the inoculum which is shake-out bacteria suspension from specimens. Add it to a test tube containing 9.0ml of the Physiological saline or the Nutrient broth or the Peptone-salt solution. Take 1 ml of this solution, add it a separate test tube containing 9.0ml of the Physiological saline or the Nutrient broth or the Peptone-salt solution.
Quantitative measurement - Plate count method -

Ensure that 1ml of each dilution is pipetted into the Petri dishes.

Add Enumeration agar to the dishes.

Quantitative measurement - Plate count method -

After incubation, count the number of colonies on the Petri dishes of dilution series on which 30 CFU to 300 CFU have appeared.

Quantitative measurement - Plate count method -

Turn the dishes upside down and incubate at 37°C for 24h to 48h.

Test result - Calculation of antibacterial activity value -

Obtain the antibacterial activity value according to the following formula.

\[
A = (\lg C_t - \lg C_0) - (\lg T_1 - \lg T_0)
\]

A : the antibacterial activity value
the common logarithm of the arithmetic average of the numbers of bacteria,

\(\lg C_t\) : obtained from 3 control specimens after an incubation
\(\lg C_0\) : obtained from 3 control specimens immediately after inoculation
\(\lg T_1\) : obtained from 3 antibacterial testing specimens after an incubation
\(\lg T_0\) : obtained from 3 antibacterial testing specimens immediately after inoculation
Table of contents

- Methods covered in this round robin test
- Test procedure
- Specified matters in the round robin test
- Test report

Specified matters in the round-robin test

1. Only *Staphylococcus aureus* (WDCM code 00193) should be used in this round robin test. This refers to the use of the strain number NBRC12732 in principle, but other strains may be used.

Strain number and culture collection organization of bacteria for this testing (example)

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Culture collection organization</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBRC 12732, NBRC 13276</td>
<td>NITE, Biological Resource Center (Japan)</td>
</tr>
<tr>
<td>ATCC 6538P, ATCC 6538</td>
<td>American Type culture Collection (USA)</td>
</tr>
<tr>
<td>FDA 209P</td>
<td>Food and Drug Administration (USA)</td>
</tr>
<tr>
<td>CIP 4.83</td>
<td>Institut Pasteur, Collection de l'Institut Pasteur (France)</td>
</tr>
<tr>
<td>DSM 799</td>
<td>German Collection of Microorganism and Cell Culture (Germany)</td>
</tr>
<tr>
<td>NCIMB 9518</td>
<td>National Collection of Industrial, Food and Marine Bacteria, Ltd. (UK)</td>
</tr>
</tbody>
</table>

Specified matters in the round-robin test

2. Incubate for 18 hours after applying inoculum to test specimens.
3. Use the plate count method for the measurement of bacterial count.
Specified matters in the round-robin test

- Use reagents and culture media listed in this table. Other reagents and culture media may be used as long as they are referred to in ISO. In this case, however, make sure to mention it in the test report.

<table>
<thead>
<tr>
<th>Specification number</th>
<th>Reagents and culture media</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1.1.2, 8.1.1.3,</td>
<td>Nutrient broth (NB)</td>
</tr>
<tr>
<td>8.1.2 Preparation of test inoculum</td>
<td></td>
</tr>
<tr>
<td>8.1.4.2 Shake-out after inoculation,</td>
<td>shake-out physiological saline</td>
</tr>
<tr>
<td>8.1.4.4 Shake-out after incubation</td>
<td></td>
</tr>
<tr>
<td>Annex C Quantitative measurement by plate</td>
<td>- Nutrient broth (NB)</td>
</tr>
<tr>
<td>count method</td>
<td>- Peptone-salt solution</td>
</tr>
<tr>
<td></td>
<td>- Physiological saline</td>
</tr>
<tr>
<td></td>
<td>(as defined in JIS L 1902: — Sodium chlorate</td>
</tr>
<tr>
<td></td>
<td>8.5g → Water 1000ml</td>
</tr>
<tr>
<td></td>
<td>pH 6.9±0.2)</td>
</tr>
<tr>
<td>same as above</td>
<td>Enumeration agar (EA)</td>
</tr>
</tbody>
</table>

Table of contents

- Methods covered in this round robin test
- Test procedure
- Specified matters in the round robin test
- Test report

Test report

The test report shall contain the following information.
- Strain number and supplier of the test bacteria
- Concentration inoculum
- Number of colonies in each petri dish
- Dilution rates of the inoculum which shake-out bacteria suspension from specimens
- Logarithmic value of viable bacteria count per sample
- Antibacterial activity value
- Judgement of test effectiveness
- Any deviation from this test method

---

**Table 1. Test using cloth specimens**

<table>
<thead>
<tr>
<th>Petri dish</th>
<th>Petri dish 2</th>
<th>Average number</th>
<th>Dilution rate</th>
<th>Concentration of inoculum (CFU/mL)</th>
<th>Strain number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---
Test report

Judgement of test effectiveness

1. Measurement of bacterial count immediately after inoculation

2. Control specimens (standard clumps)

3. Test specimens

4. Difference in the number of bacteria immediately after inoculation and after incubation, respectively, shall be less than two.

Test report

Judgement of test effectiveness

1. The test inoculum shall be $1 \times 10^5$ CFU/ml.

2. The difference in the common logarithm in the number of bacteria for the three control specimens immediately after inoculation and after incubation, respectively, shall be less than one.

Test report

Judgement of test effectiveness

The growth value obtained according to the following formula shall be equal or more than 1.0.

$\text{Growth value} = \frac{\text{log}_{10} N_2 - \text{log}_{10} N_1}{N_1}$

where:
- \( N_1 \): Number of bacterial in control specimens
- \( N_2 \): Number of bacterial in test specimens

The judgement of test effectiveness is as follows:

- If $\text{Growth value} \geq 1.0$, then test effective.
- If $\text{Growth value} < 1.0$, then test not effective.

Test report

Judgement of test effectiveness

1. Measurement of bacterial count immediately after inoculation

2. Control specimens (standard clumps)

3. Test specimens

4. Difference in the number of bacteria immediately after inoculation and after incubation, respectively, shall be less than two.
1. The kind of antibacterial and a deodorant agents
1.1. About antibacterial agent

Antibacterial agent (抗菌劑)
- The chemical substance made by artificial composition.

Antibiotics (抗生素)
- The chemical substance which a microbe formed.

Antibacterial processing
- JIS L 1902: control a bacterial increase on the fiber by decreasing bacteria.

2. Textile production process
2-1. From raw cotton to yarn
2-2. From yarn to textile

3. Antibacterial processing method of the fiber

4. About around robin test

CONTENTS
1. The kind of antibacterial agents
   1.1. About antibacterial agent
   1.2. Classification of antibacterial agents
      (1) Classification by the purpose
      (2) Classification by the material

2. Textile production process
   2-1. From raw cotton to yarn
   2-2. From yarn to textile

3. Antibacterial processing method of the fiber

4. About around robin test

Antibacterial agents
- Environmental antibacterial agents
  - for microbes in environment
- Living body antibacterial agents
  - for the microbe which infected a person.

Antibacterial processing of the fiber
  = Environmental antibacterial
Antibacterial agents

<table>
<thead>
<tr>
<th>Environmental</th>
<th>Living Body</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (60~85% Ethanol, Isopropanol)</td>
<td></td>
</tr>
<tr>
<td>Phenolic compounds (Hexachlorophene, Chlorhexidine etc.)</td>
<td></td>
</tr>
<tr>
<td>Positive ion surfactant</td>
<td></td>
</tr>
<tr>
<td>Quaternary ammonium salt compound</td>
<td>(Benzalkonium chloride)</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>3% Hydrogen peroxide</td>
</tr>
<tr>
<td>Ethylene oxide gas</td>
<td>Iodophors (Povidone Iodine)</td>
</tr>
</tbody>
</table>

1. The kind of antibacterial and a deodorant agents
1.2. Classification of antibacterial agents
(2) Classification by the material

- Inorganic compounds
  - Type:
    - Metal ions Compounds
    - Photo catalyst (TiO₂)

- Organic compounds
  - Type:
    - Chemical compounds
    - Natural products

Characteristics of inorganic compounds
◆ Good point
  - stable at the high temperature
  - wide antibacterial spectrum
◆ Weak Point
  - Antibacterial effect of the quantity of agents is lower than an organic compounds.

Characteristics of organic compounds
◆ Good point
  - chemical compound = cheap, high effect (small quantities)
  - natural product ⇒ many generally safe things
◆ Weak Point
  - low heat resistance
  - antibacterial spectrum is small
  - resistant bacteria may emerge

As for the antibacterial processing of textiles, a lot of antibacterial agents of the organic chemical compounds are used from cost, stability.
2. Textile production process

2.1. From raw cotton to yarn

<table>
<thead>
<tr>
<th>Characteristics of antibacterial agents</th>
<th>Heat resistance</th>
<th>Antibacterial activity</th>
<th>Stain</th>
<th>Cost performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>As, Ca, Zn</td>
<td>0</td>
<td>0</td>
<td>0</td>
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Natural fiber: Cotton, Wool, Silk

Polyester, Acrylic, etc.
2.1. From raw cotton to yarn

(1) Opening and picking process (混打線)

It is performed in fore-spinning process of the spinning. After mixing the different cotton lump of the kind, and untying a cotton lump using a beater (body of rotation), and having removed cotton lump impurities, it is done by the belt-shaped fiber assembly called the lap.

(2) Carding process (捫絲工程)

The sheet-shaped lap processed in the mixing and blowing process is combed using the carding machine to separate the fibers and remove fine dust and short fibers. Remaining long fibers are aligned nearly parallel and collected to be processed into the string-shaped "carded sliver."

(3) Combing (鏡梳線工程)

The carded sliver is further combed to remove short fibers and dust that could not be removed in the carding process. Fibers are then arranged parallel to obtain uniform combed sliver. This process is essential to manufacture uniform, high-quality yarn.

(4) Drawing (線條工程)

Six to eight slivers after the carding or combing process are gathered and elongated to six to eight times their original length using a drawing machine to straighten and remove uneven thickness from the fibers. This process transforms fibers into string-like "drawn sliver."
2-1. From raw cotton to yarn

(5) Roving

Since the drawn sliver is too thick to produce yarn directly, it is further elongated using a roving machine. Twisting is first applied to fibers in this process to obtain the green yarn, which is wound onto a bobbin.

2-1. From raw cotton to yarn

(6) Spinning

In the fine spinning process, the last of the main spinning processes, the green yarn resulting from the roving process is further elongated to obtain a desired thickness and then twisted. The final product, or the finished yarn, is wound on a bobbin.

2-1. From raw cotton to yarn

(7) Winding

The winding process involves rewinding the finished yarn onto bobbins into the cheese or cone according to its purpose.

2. Textile production process

2-2. From yarn to textile
2-2. From yarn to textile

(1) Warping

Cheese/cones are set on a warping machine to wind the predetermined length and number of yarns onto the predetermined number of warping beams under constant tension.

Ref. KUROSE CO., LTD.

(2) Sizing

Because the hairiness of the yarn affects the weaving, it is necessary to decrease the hairiness of the yarn. The warping beams of the required number of warp of the final textile are piled up for rewinding on beams after sizing and drying.

Ref. T-Tech Japan Corp.

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2-2. From yarn to textile

(3) Drawing-in

Drawing-in

To prepare for setting beams on a loom, warps are routed in the order of droppers, healds and guide bars.

Ref. MARUMATSU SHOKUFU Co., Ltd.

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2-2. From yarn to textile

(4) Weaving

Prepared beams are set on a looming frame to weave a textile in the following five motions:

1. Shedding: two groups of warps are opened to let the weft pass through.
2. Picking: The weft is inserted between two groups of warps.
3. Beat-up: Pushing the newly inserted yarn back into the fell using reed.
4. Let-off: The warp yarns are unwound from the warp beam.
5. Take-up: The woven fabric is wound on the cloth beam.
2. Textile production process

2-3. Dyeing, finishing process of cotton fabrics

Preparation process
- Stability and dimensions stability improve when removing unnecessary ingredients (pectin, wax, sizing agent, etc.) attaching to a gray fabric.

Dyeing process
- Mainly, dye of the cotton fiber is reaction and VAT dye. As for the adhesion of the dye of the cotton fiber, the reaction dye occurs by chemical bond and the VAT dye occurs by physical adsorption.

Finishing process
- This process is control the texture of the fiber with a softener and give various functions by function agents.

Preparation process

- Singeing
  - To remove paste
  - Refinement
  - Bleaching
  - Mercerized

Hairiness of the textile surface is removed by the flame of the gas burner.

Before

After
**Preparation process** To remove paste, Refinement, Bleaching

To remove paste, refinement, bleaching. Cotton textiles usually perform these processes consecutively.

**About a round robin test**

**Purpose**
Each testing institution of the participation economy conducts round robin test for antibacterial and a deodorant. The test data is performed a statistical analysis of and knows the performance of each testing institution. In addition, the result is informed each testing institution of and assumes it an examination document.

**Examination**
Antibacterial and Deodorant Test

**Test sample**
- Textile of the Cotton / PET mixed spinning
- A) Quaternary ammonium salt-based agent
- D) Surfactant-based agent

**Test method**
A method established in SEK mark certification standard

**Test target**
- A) S.aureus
- D) ammonia