สถาบันอาหาร
กระทรวงอุตสาหกรรม

PROFICIENCY TESTING

LABORATORY ACCREDITATION
BLA-DSS

PTP
No.0002

Microbiological Analysis

NFI-PTM 01-2019: Total Plate Count (CFU/g) in Freeze Dried Chicken

January 2019

LABORATORY SERVICES DEPARTMENT

national food institute
ministry of industry

Proficiency Testing Report

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SUMMARY

This summarizes the microbiological proficiency testing program of NFI-PTM 01-2019: Total Plate Count (CFU/g) in Freeze Dried Chicken. There were 70 laboratories participating in this program. The test materials were quality controlled to ensure that consistent levels were achieved throughout the batch by conducting homogeneity test prior to dispatch. Stability testing was conducted at the end of shelf life which was the deadline for all laboratories to run the test - to ensure the integrity of the test materials.

The participant's results were 70 laboratories which were assessed statistically in the form of a robust Z-Score for performance evaluation for each laboratory. The overall competency evaluation was that there were 56 laboratories (80.0%) had satisfactory performance with $|Z| \leq 2.00$, while 10 laboratories (14.3%) had questionable performance with $2.00 < |Z| < 3.00$, and 4 laboratories (5.7%) had unsatisfactory performance with $|Z| \geq 3.00$.

This proficiency testing program accomplished with smooth operation as expected, and every participating laboratory benefits and acquires valuable information for advancement whatever the performance result is.

1. INTRODUCTION

1.1 Proficiency Testing

Proficiency testing (PT) is a valuable tool for quality assurance of testing and measurement. It is a requirement of accreditation to ISO/IEC 17025 that laboratories take part in proficiency testing schemes. PT involves the use of interlaboratory comparisons for determination of laboratory performance with the purpose of producing laboratory results of greater reliability. Whatever the performance result is, every participating laboratory benefits and acquires valuable information for further advancement in all degree of their current performance.

For the improvement and satisfaction to the requirement of ISO/IEC 17025 of laboratories, hence the Division of Proficiency Testing Laboratory, Department of Laboratory Services, National Food Institute (NFI) provides PT program to follow the international standard ISO/IEC 17043: 2010, Conformity Assessment – General Requirements for Proficiency Testing. This program was named "Microbiological Analysis NFI-PTM 01-2019: Total Plate Count (CFU/g) in Freeze Dried Chicken" with the focus on total plate count since it is one of the most common tests.

To ensure performance confidentiality of all participants, a series of Laboratory Number is used in place of participant's name. A unique Laboratory Number is assigned to each laboratory.

NFI offers Internet access for proficiency testing at 'http://pt.nfi.or.th' for fast, convenient and easy access for participants to register, submit form / data / test result / request, view other information, and communicate with the provider. All participants are encouraged to utilize all on-line features and more for complete and clear action for their own advantage.

1.2 Total Plate Count

Enumeration of microorganisms by total plate count is one of the most common methods used to indicate microbiological quality in food with the commonly practice conditions: 30 - 35 ^{o}C and 48 - 72 hours for incubation. The total plate count (TPC) is also called the aerobic plate count, standard plate count (SPC), total viable count (TVC) or mesophilic count represents the number of colony forming units (CFU) per g (or per mL) of growing microorganisms such as bacteria, yeast and mold on a non-specific solid bacteriological growth medium under the specified conditions. Temperature, incubating time, medium type, and any other conditions used in the enumeration varies

upon reference standards and laboratory objectives. Laboratories participating in the program choose their own routine conditions.

2. TEST MATERIALS

2.1 Sample Preparation

Test materials were produced in bulk by blending dried chicken, powdering, and mixing it well. Tight-sealing-cap plastic tubes containing 2 grams of the chicken powder were prepared, and each was labeled with ID numbers and stored at 2-8°C for homogeneity test, and further steps. For enumeration calculation, the test material weight of 2 grams was equivalent to 10 grams of the initial chicken weight.

2.2 Homogeneity

To insure that consistent levels of organisms were achieved throughout the batch of test materials, homogeneity test was conducted on 3 January 2019. The random number generator from the website 'random.org' was used to randomize the order of the samples. Ten out of all the tubes were randomly picked for homogeneity test by carrying out total plate count (CFU/g) in duplicate prior to dispatch.

2.3 Stability

To ensure the constancy of the test, materials not being diminished over time and the transport effect, stability testing was conducted at the end of shelf life. Therefore, five sets of the test materials at NFI were packaged using the same types of materials (boxes, ice packs, etc.) and conditions (temperature, length of time, etc.) as those delivered; leaving the selected-random sample in the box for 24 hours, and then keeping at 2-8°C until the final test or after this date. On 16 January 2019, the test materials were examined in duplicate for enumeration of total plate count (CFU/g).

The work on homogeneity and stability tests was conducted by the supporting laboratory – Division of Microbiological Laboratory, National Food Institute (ISO/IEC 17025: 2005: DMSC Acc. No. 1005/42). The homogeneity and stability data were shown in Table 3 in the Appendix.

2.4 Sample Distribution

Upon completion of homogeneity test, foam boxes of test materials with ice packs, and documents were dispatched on 9 January 2019 to each participant by an express logistics company with 24-hour guaranteed delivery. Documents enclosed in the box were as follows:

1) Letter of Introduction
2) Test Instruction Sheet
3) Receipt Form
4) Results Form
5) Technical Form
6) On-line Instruction Sheet

Note:

- Filling on-line preferred for 3), 4) and 5).
- Using test methods (materials, procedures, conditions, etc.) consistent with each laboratory routine practice.
- One tube of test material delivered for each participant.

Participants were required to report and submit their enumeration of the test material in CFU/g by the closing date – "23 January 2019".

3. REPORTING

Having finished the calculation of the test result, each participating laboratory, then submitted its test result by the closing date for analysis, and for the summary report to be made. With all these data, together with those received through PT provider's quality control, statistical analysis for homogeneity test, stability test, and others were carried out according to the standard ISO 13528: 2015.

The statistics summarizes are given in Table 1. Table 2 summarizes the result of participants. Technical data of methods used by each laboratory in relation to accreditation, reference, and others are summarized in Table 4 in the Appendix.

Interim report and final report could be uploaded to the website pt.nfi.or.th within two weeks and one month, respectively. After the closing date, while hard copies of interim and final report could be sent to participants with no Internet access by registered mail.

4. STATISTICAL EVALUATION OF RESULTS

Statistical use for this proficiency testing program is robust Z-Score that is based on ISO 13528: 2015.

4.1 Calculation of the assigned value, x_{pt} and standard uncertainty of the assigned value, $u(x_{pt})$

4.1.1 Assigned value, x_{pt} (assigned value for proficiency assessment) was set as the consensus of the results submitted by participants. The procedure used to derive this consensus involved some or all of the following:

4.1.1.1 Log transformation of participant results from CFU/g to $\log_{10}$CFU/g.

4.1.1.2 Removal of data that was not considered valid.

4.1.1.3 Derivation of the robust mean (x^*) of the final data set using a robust statistical procedure that calculate by Algorithm A.

4.1.2 Assessment of the standard uncertainty of the assigned value, $u(x_{pt})$

$$u(x_{pt}) = 1.25 \times \frac{s^*}{\sqrt{p}} \qquad \text{.....(1)}$$

Where s^* is robust standard deviation calculated by Algorithm A

and p is number of result.

When the assigned value is calculated from the formulation of the proficiency test item, the standard uncertainty $u(x_{pt})$ of the assigned value is calculated according to equation (1). The standard uncertainty of the assigned value is usually compared with a criterion (2), that $u(x_{pt})$ shall be smaller than $0.3\sigma_{pt}$, then the uncertainty of the assigned value is negligible and need not be included in the interpretation of the results of the proficiency test.

$$u(x_{pt}) < 0.3\sigma_{pt} \qquad \text{.....(2)}$$

4.2 Standard deviation for proficiency assessment, σ_{pt}

The standard deviation for proficiency assessment used here has been estimated from the previous standard deviation of NFI-PT microbiological enumeration program (CFU/g) and the advisory group has agreed that an appropriate value is 0.300 $\log_{10}$CFU/g. This value for σ_{pt} has been used to calculate Z-Score in this assessment.

4.3 Calculation of robust Z-Score

The *Z-Score* for a proficiency test result is calculated as follows *Z-Score* equation (3)

$$Z\text{-}Score = \frac{(log_{10}x_i - log_{10}x_{pt})}{\sigma_{pt}} \quad \text{.....(3)}$$

Where x_i is the participant's reported result,

x_{pt} is the assigned value

and σ_{pt} is the standard deviation for proficiency assessment.

4.4 Z-Score assessment criteria

$|Z| \leq 2.00$ is Satisfactory

$2.00 < |Z| < 3.00$ is Questionable

$|Z| \geq 3.00$ is Unsatisfactory

5. REFERENCES

ISO/IEC 17043: 2010. Conformity assessment – general requirements for proficiency testing.

ISO 13528: 2015. Statistical methods for use in proficiency testing by interlaboratory comparison.

Maturin, L.(ret.) and J.T. Peeler.(ret.). 1998. Chapter 3 – Aerobic Plate Count, *in* the Bacteriological Analytical Manual – *Online* (January 2001), Food and Drug Administration. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm (February 2019).

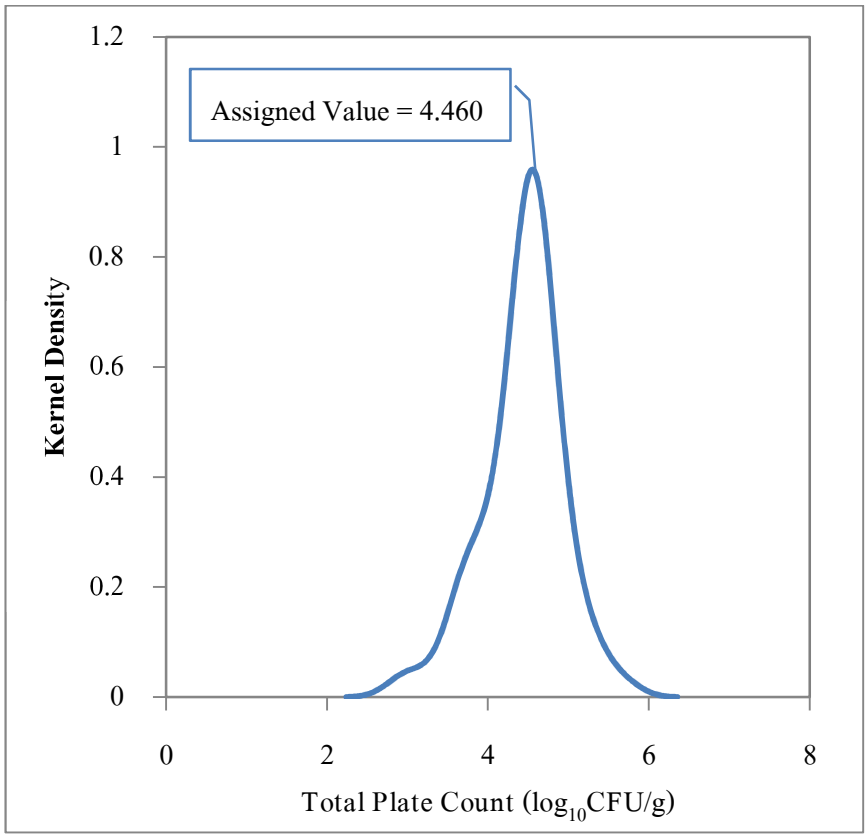
6. RESULTS

Table 1 Statistic summary

Statistic Data					Standard Deviation for Proficiency Assessment	
Number of Result	Assigned Value, x_{pt} ($\log_{10}$CFU/g)	Robust Standard Deviation, **s***	Standard Uncertainty, $u(x_{pt})$	Coefficient of Variation, (%CV)	Derived from	σ_{pt}
70	4.460	0.421	0.063	9.4	Retrospective data	0.300

Note: σ_{pt} derived from retrospective data that an appropriate value is 0.300 $\log_{10}$CFU/g.

Figure 1 Kernel density plot for participant results



Note: The Kernel density show distribution of the 70 participant results.

Table 2 Result summary of participants' Z-Score for total plate count (CFU/g) in freeze dried chicken

Laboratory Number	Result CFU/g	Result Log_{10}CFU/g	Z-Score
1	7400	3.869	-1.97
2	3300	3.519	**-3.14**
3	5,000	3.699	**-2.54**
4	17000	4.230	-0.77
5	44000	4.643	0.61
6	18333	4.263	-0.66
7	103,000	5.013	1.84
8	21000	4.322	-0.46
9	54,000	4.732	0.91
10	480,000	5.681	**4.07**
11	14,000	4.146	-1.05
12	35,000	4.544	0.28
13	60000	4.778	1.06
14	32,000	4.505	0.15
15	22000	4.342	-0.39
16	6,400	3.806	**-2.18**
17	64000	4.806	1.15
18	45000	4.653	0.64
19	16,000	4.204	-0.85
20	6,000	3.778	**-2.27**
21	34000	4.531	0.24

Note: Z-Score values outside the satisfactory range are shown in bold.

Table 2 Result summary of participants' Z-Score for total plate count (CFU/g) in freeze dried chicken (continued)

Laboratory Number	Result CFU/g	Result Log_{10} CFU/g	Z-Score
22	42000	4.623	0.54
23	7800	3.892	-1.89
24	29500	4.470	0.03
25	34000	4.531	0.24
26	40000	4.602	0.47
27	3800	3.580	**-2.93**
28	35000	4.544	0.28
29	52,000	4.716	0.85
30	28,300	4.452	-0.03
31	820	2.914	**-5.15**
32	1,400	3.146	**-4.38**
33	36,000	4.556	0.32
34	25,000	4.398	-0.21
35	37,000	4.568	0.36
36	40,000	4.602	0.47
37	46000	4.663	0.68
38	76000	4.881	1.40
39	25,600	4.408	-0.17
40	9100	3.959	-1.67
41	11409	4.057	-1.34
42	35,000	4.544	0.28

Note: Z-Score values outside the satisfactory range are shown in bold.

Table 2 Result summary of participants' Z-Score for total plate count (CFU/g) in freeze dried chicken (continued)

Laboratory Number	Result CFU/g	Result Log_{10} CFU/g	Z-Score
43	35000	4.544	0.28
44	30,000	4.477	0.06
45	35000	4.544	0.28
46	100000	5.000	1.80
47	62000	4.792	1.11
48	80,000	4.903	1.48
49	126,500	5.102	**2.14**
50	32,000	4.505	0.15
51	210,000	5.322	**2.87**
52	24,000	4.380	-0.27
53	4,200	3.623	**-2.79**
54	22000	4.342	-0.39
55	159000	5.201	**2.47**
56	216000	5.334	**2.91**
57	56000	4.748	0.96
58	40000	4.602	0.47
59	38,000	4.580	0.40
60	48,000	4.681	0.74
61	5400	3.732	**-2.43**
62	24,000	4.380	-0.27
63	78,000	4.892	1.44

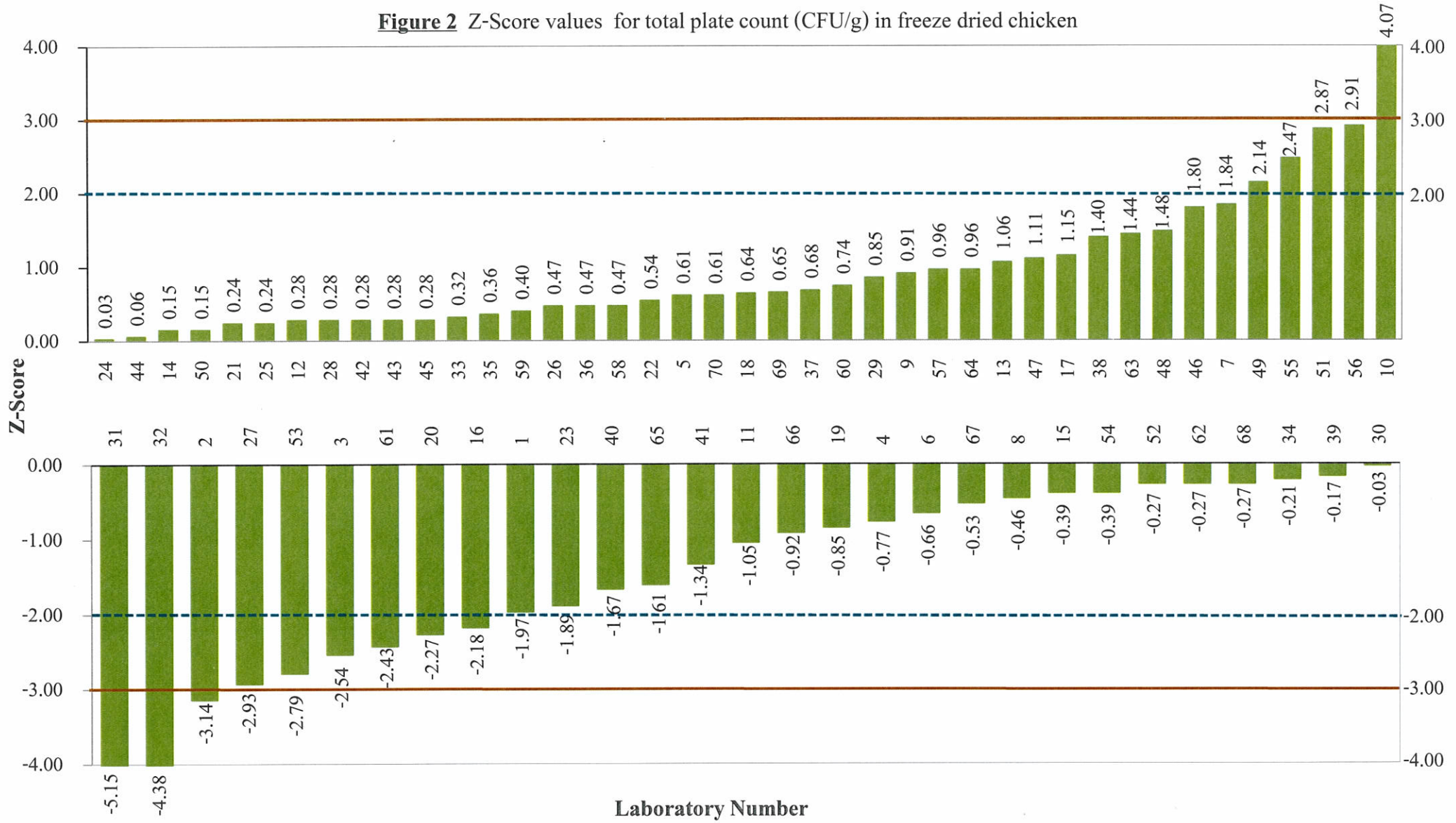
Note: Z-Score values outside the satisfactory range are shown in bold.

Table 2 Result summary of participants' Z-Score for total plate count (CFU/g) in freeze dried chicken (continued)

Laboratory Number	Result CFU/g	Result Log_{10} CFU/g	Z-Score
64	56,000	4.748	0.96
65	9,500	3.978	-1.61
66	15,300	4.185	-0.92
67	20,000	4.301	-0.53
68	24,000	4.380	-0.27
69	45,330	4.656	0.65
70	44,000	4.643	0.61

Note: Z-Score values outside the satisfactory range are shown in bold.

Figure 2 Z-Score values for total plate count (CFU/g) in freeze dried chicken



7. METROLOGICAL TRACEABILITY

The provider requires participants to report their test results in CFU/g. The traceability of a measurement result in microbiological testing as made available from standard method.

The laboratory shall have a program for calibrating, verifying the performance of all critical equipment and certified reference standards, traceable to national standards.

8. TECHNICAL COMMENTS

Participant having questionable and unsatisfactory performance are recommended to find the cause of their errors and correct them. For this proficiency testing program, there are three main viewpoints to be considered by those in need of improvement:

8.1 Analytical technique

The laboratory must be careful to all details in the testing process, such as colony count (Be careful about the appearance of colonies that similar to the sample.), incubation temperature, and incubation time. Furthermore, the laboratory should be prepared sample following by the instruction's document of PT provider.

8.2 Sample mixing

Mixing is the major step that is used to evenly spread the microorganism. If the laboratory is not carefully concerned in this step, the quantity of microorganism will incur either in the less or more than the actual quantity.

8.3 Calculation of the test result and the data transfer

The laboratory must carefully calculate the test result, e.g. multiplication of the dilution and checking of the data transfer before reporting.

NFI is pleased to provide further detail, and welcome any discussion regarding the proficiency testing.

9. CONTACT DETAILS

Upon receiving the final report, if there is any question, complaint and appeal regarding the proficiency testing assessment incurs, written notification should be submitted to the project coordinator within thirty days by one of the following channels:

Mail: Miss Thatsanee Kansan (Project coordinator)
Division of Proficiency Testing Laboratory,
Department of Laboratory Services, National Food Institute,
2008 Soi Arun Ammarin 36, Arun Ammarin Rd., Bangyeekhan, Bangphlat, Bangkok 10700 THAILAND.

Fax number: 0 2422 8554

E-mail: pt@nfi.or.th

Website: Contact-Us Form at http://pt.nfi.or.th

10. APPENDIX

Table 3 Homogeneity and stability data

Total Plate Count ($\log_{10}$ CFU/g)*					
Sample No.	**Homogeneity (y_1)**		**Sample No.**	**Stability (y_2)**	
	Replicate 1	**Replicate 2**		**Replicate 1**	**Replicate 2**
1	4.380	4.342	1	4.447	4.491
2	4.477	4.580	2	4.477	4.505
3	4.447	4.431	3	4.613	4.505
4	4.531	4.447	4	4.415	4.531
5	4.491	4.447	5	4.342	4.230
6	4.531	4.362	-	-	-
7	4.462	4.301	-	-	-
8	4.505	4.431	-	-	-
9	4.477	4.431	-	-	-
10	4.531	4.462	-	-	-
mean	4.453			4.456	
S_s	0.019			-	
$\lvert\bar{y}_1 - \bar{y}_2\rvert$	-			0.003	
σ_{pt}**	0.300			0.300	
0.3 σ_{pt}	0.090			0.090	
$S_s \leq 0.3\sigma_{pt}$	**PASS**			-	
$\lvert\bar{y}_1 - \bar{y}_2\rvert \leq 0.3\sigma_{pt}$	-			**PASS**	

Note: * FDA-BAM *Online*, 2001 (Chapter 3)

** σ_{pt} derived from retrospective data that an appropriate value is 0.300 $\log_{10}$CFU/g.

Table 4 Technical information in relation to the methods

Accredited	**Laboratory Number**
Yes	1 3 7 9 15 17 22 23 26 27 29 33 34 40 42 43 44 45 47 48 49 52 54 55 58 62 64 67
No	2 4 5 6 8 10 11 12 13 14 16 18 19 20 21 24 25 28 30 31 32 35 36 37 38 39 41 46 50 51 53 56 57 59 60 61 63 65 66 68 69 70
Reference	**Laboratory Number**
AOAC	2 4 7 10 11 12 16 23 24 25 26 28 30 33 34 48 50 55 63 64 66 67 68 69
AOAC (2016) 990.12 and APHA (2015)	43
APHA	54 58
FDA-BAM	1 5 6 8 9 14 15 17 18 19 20 21 22 27 29 31 32 35 36 37 38 39 40 41 42 44 45 46 47 49 51 52 53 57 59 60 61 62 65 70
ISO 4833	3 56
MHLW, Japan	13

Table 4 Technical information in relation to the methods (continued)

Media: Growth Media	Laboratory Number
Petrifilm	2 4 7 10 11 12 24 25 26 28 30 33 34 48 50 55 66 67 68 69
Petrifilm 3M AC and PCA	43
Plate Count Agar	1 3 5 6 8 9 13 14 15 16 17 18 19 20 21 22 23 27 29 31 32 35 36 37 38 39 40 41 42 44 45 46 47 49 51 52 53 54 56 57 58 59 60 61 62 63 64 65 70
Incubation Temperature (°C)	**Laboratory Number**
30	3
32	19
35	1 2 4 5 6 8 9 10 11 12 13 14 15 16 17 18 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 61 62 64 65 66 67 68 70
36	69
37	7 60 63

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Proficiency Testing Provider Accreditation

ISO/IEC 17043: 2010: BLA-DSS Acc. No. PTP-0002

Approved by

(Miss Preeyaporn Jaengkarnkit)

Technical Manager

21 February 2019

สถาบันอาหาร
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ติดต่อสอบถามเพิ่มเติมได้ที่

แผนกทดสอบความชำนาญ ฝ่ายบริการห้องปฏิบัติการ

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