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Verification of Total Plate Count Testing Method on Dairy Goat Milk Referring to SNI 2897: 2008

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ABSTRACT
Background: A method must be verified and validated because each laboratory has varying conditions. This diversity can occur due to differences in facilities, personnel competence, equipment and chemicals used. In addition, accredited testing laboratories have an obligation to carry out method validation and verification processes before implementing the method. Objective: In this study, the ALT testing method for dairy goat milk was verified using accuracy and precision test parameters from samples with low, medium, high and uncontaminated concentrations. Method: This research uses descriptive research, namely describing the results of verification of the ALT testing method on fresh goat's milk which refers to SNI 2897:2008. Results: The SNI 2897:2008 ALT testing method on dairy goat milk for all microbial concentrations can be applied at the Denpasar Ministry of Health Health Polytechnic Integrated Laboratory because the RSD and Percent Recovery values do not exceed the limits set in SNI 2897:2008. Conclusion: The Total Plate Number testing method according to SNI 2897:2008 with a dairy goat's milk matrix can be applied as a testing method at the integrated laboratory of the Health Polytechnic of the Ministry of Health, Denpasar.

Keywords: *verificationi, TPC, milk, SNI*

ABSTRAK
Latar Belakang : Suatu metode harus diverifikasi dan divalidasi karena setiap laboratorium memiliki kondisi yang beragam. Keragaman tersebut dapat terjadi karena perbedaan sarana, kompetensi personel, peralatan, dan bahan kimia yang digunakan. Selain itu, laboratorium pengujian yang telah terakreditasi memiliki kewajiban untuk melakukan proses validasi dan verifikasi metode sebelum implementasi metode. Tujuan : Pada penelitian ini, dilakukan verifikasi metode pengujian ALT pada susu kambing perah dengan parameter uji akurasi dan presisi dari sampel dengan konsentrasi rendah, sedang, tinggi dan tidak terkontaminasi. Metode : Penelitian ini menggunakan penelitian deskriptif, yaitu menggambarkan hasil verifikasi metode pengujian ALT pada susu kambing segar yang merujuk pada SNI 2897:2008. Hasil : Metode pengujian ALT SNI 2897:2008 pada susu kambing perah semua konsentrasi mikroba dapat diterapkan di Laboratorium Terpadu Poltekkes Kemenkes Denpasar karena nilai RSD dan Persen Recovery tidak melebihi batas yang ditetapkan dalam SNI 2897:2008. Kesimpulan : Metode pengujian Angka Lempeng Total sesuai SNI 2897:2008 dengan matriks susu kambing perah dapat diterapkan sebagai metode pengujian di Laboratorium terpadu Poltekkes Kemenkes Denpasar.

Kata kunci: *verifikasi, TPC, susu, SNI*

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INTRODUCTION

Standardization of sample testing is crucial for laboratories serving the general public. Laboratories play a vital role in research, education, and community service activities. Recognition of a laboratory's capability in testing a particular parameter can be achieved through a series of verification activities for a testing method. Testing laboratories should conduct verification activities for each method used in serving the public.

A method must be verified and validated because each laboratory operates under different conditions. These variations may arise due to differences in facilities, personnel competencies, equipment, and chemicals used. Generally, validation and verification of methods are carried out before a testing method is used in routine analysis within the laboratory. Furthermore, accredited testing laboratories are required to perform method validation/verification before method implementation (1)(2).

Currently, the consumption of fresh goat milk is gaining popularity among the public. However, most dairy goat farms in Indonesia are still managed on a small scale using simple methods (3). Fresh goat milk can be a source of transmission for various pathogenic bacteria such as *Staphylococcus aureus, Listeria monocytogenes, Campylobacter spp.*, and coliform bacteria (4)(5)(6). To date, fresh goat milk has received little attention from the government or other relevant institutions concerning quality control, safety, and hygiene (7)(5).

The verified Total Plate Count (TPC) testing method at the Integrated Laboratory of Poltekkes Denpasar has been used to determine microbial counts in boiled eggs. The TPC testing method refers to SNI 2897:2008, which is the testing method for microbial contamination in meat, eggs, milk, and their processed products. Currently, no verified analytical method is available for microbial count testing in dairy goat milk (8). This study aims to verify the TPC testing method for dairy goat milk by evaluating accuracy and precision parameters for samples with low, medium, high, and uncontaminated concentrations. The verification results will be implemented as one of the testing services at the Integrated Laboratory of Poltekkes Kemenkes Denpasar. This effort serves as a part of public service to support the critical role of the Tri Dharma of Higher Education.

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MATERIALS AND METHODS

This study employs a descriptive research method to describe the verification results of the TPC testing method on fresh goat milk, referring to SNI 2897:2008 (9). The goat milk samples were obtained from a goat farm located at Jl. Maruti No.5, Pemecutan Kaja, North Denpasar District, Denpasar City, and sample testing was conducted at the Integrated Laboratory of Poltekkes Kemenkes Denpasar.

The equipment used includes an analytical balance (Radwag), a

blender (Myako), a graduated pipette (Iwaki Pyrex®) 25 ml, a micropipette (Socorex) 100µl-1000µl and tips, a ball pipette (D&N), a measuring cylinder (Iwaki-Pyrex) 250 ml, sterile Petri dishes, test tubes (Iwaki), a biosafety cabinet (Biobase), an incubator (Esco), an autoclave (Tomy SX-500), a glass funnel, a glass stirrer, a magnetic stirrer, a colony counter, a sterile inoculation loop, a McFarland densitometer, a vortex mixer, a water bath, and a hot plate magnetic stirrer. The materials used include fresh goat milk, Plate Count Agar (PCA), and Buffered Peptone Water (BPW).

Each treatment group (uncontaminated, low, medium, and high concentration) required 80 ml of fresh goat milk. Precision was determined by calculating the relative standard deviation (RSD), while accuracy was assessed by calculating the % recovery from TPC testing of fresh goat milk using seven repetitions (10). The study was conducted from February to August 2024.

Fresh goat milk samples, each with a volume of 80 ml, were placed in four Erlenmeyer flasks (high, medium, low concentrations, and uncontaminated) containing 720 ml of BPW. The samples were then divided into eight Erlenmeyer flasks, each containing 90 ml for high concentration, eight Erlenmeyer flasks for medium concentration, eight for low concentration, and eight for uncontaminated samples. All samples were sterilized at 121°C for 15 minutes.

Bacterial concentrations in 10 ml of sterile distilled water (bacterial stock) were prepared by inoculating one loop of 18-24-hour-old agar culture into sterile distilled water. The turbidity was adjusted to the McFarland 0.5 standard (10^8 CFU/ml). Bacterial suspension dilutions of 10^3 - 10^7 CFU/ml were prepared. For bacterial spiking into sample matrices, a 10^7 CFU/ml bacterial suspension was added to high concentration samples, 10^5 CFU/ml to medium concentration samples, 10^3 CFU/ml to low concentration samples, and sterile distilled water to uncontaminated samples. All samples were then processed according to SNI 2897:2008 standards.

TPC testing based on SNI 2897:2008 involves several steps. First, 25 ml of fresh goat milk (uncontaminated, low, medium, and high concentrations) is measured aseptically and added to 225 ml of 0.1% sterile BPW, creating a 10^{-1} dilution. Next, 1 ml of the 10^{-1} dilution is transferred using a sterile pipette into 9 ml of BPW to obtain a 10^{-2} dilution. Further dilutions (10^{-3}, 10^{-4}, 10^{-5}, etc.) are prepared similarly as needed.

Then, 1 ml of each dilution suspension is placed into duplicate sterile Petri dishes. Subsequently, 15-20 ml of PCA, cooled to 45°C ± 1°C, is added to each Petri dish. To mix the sample solution and PCA medium thoroughly, the dishes are gently rotated forward and backward or in a figure-eight motion and left to solidify. Incubation is performed at 34°C to 36°C for 24-48 hours with inverted plates. Specifically for dairy products, incubation is conducted at 32°C ± 1°C for 24-48 hours with inverted plates (11)(12).

RESULTS AND DISCUSSIONS

The verification process was divided into four treatment groups: samples without spike addition, samples with low, medium, and high concentrations, all tested in duplicate (13)(14). Colonies growing on the media were observed and counted using a colony counter. The results of the observations and colony counts are presented in the table below.

Table 1. Results of Bacterial Colony Count in Goat Milk Samples

No	Colony Count							
	Uncontaminated		Low Microbial Concentration (10^{-1} Dilution)		Medium Microbial Concentration (10^{-2} Dilution)		High Microbial Concentration (10^{-5} Dilution)	
	1	2	1	2	1	2	1	2
1	0	0	7	7	153	183	106	116
2	0	0	3	3	165	169	128	118
3	0	0	9	8	164	203	115	102
4	0	0	7	6	148	209	131	125
5	0	0	5	6	150	174	111	126
6	0	0	4	3	162	159	125	128
7	0	0	5	5	158	180	116	121
8	0	0	6	6	105	95	105	115

In Table 1, it can be seen that the dairy goat milk samples with spike addition exhibit varying colony growth from repetition 1 to repetition 8. This variation occurs because the analyte used in the test is a living organism, whose growth can be influenced by factors such as nutrients, pH, oxygen, temperature, and incubation time, making it difficult to control. Nevertheless, the variation in colony numbers is considered relatively stable as it remains within the same order of magnitude, thus not significantly affecting the results. The required performance characteristics that must be calculated for the verification of quantitative microbiological methods in food and feed matrices are precision and accuracy (15).

A. Precision

Precision refers to the degree of agreement between individual test results when the method is repeatedly applied to multiple samplings of a homogenized sample. The accuracy of an analytical method is usually expressed as the relative standard deviation (CV or Coefficient of Variation) from a series of measurements (16)(17) .The recommended procedure for estimating inter-laboratory precision is the Relative Standard Deviation (RSD):

$$RSD = \sqrt{\frac{\sum_{i=1}^{i=n}[(\log a_i - \log b_i/x_i)]^2}{2p}}$$

Keterangan :

$\frac{(\log a_i - \log b_i)}{x_i}$ = The relative difference between the logarithm results of duplicates

i = 1,2,…n

p = number of duplicate determinations

Based on the RSD formula above, the RSD and CV values for dairy goat milk samples with low, medium, and high microbial concentrations are as follows:

Table 2
RSD and CV Values of Dairy Goat Milk Samples.

No	Dairy Goat Milk Sample	RSD Value	CV Value
1	**Low microbial concentration**	0,0259	2,59%
2	**Medium microbial concentration**	0,0125	1,25%
3	**High microbial concentration**	0,0037	0,37%

Based on the table above, the RSD value for low microbial concentration is 0.0259, with a CV of 2.59%. For medium concentration, the RSD value is 0.0125, and the CV is 1.25%. For high concentration, the RSD value is 0.0037, and the CV is 0.37%. The acceptable RSD criterion used as a reference is < 5%. Therefore, the TPC testing method based on SNI 2897:2008 for dairy goat milk with low, medium, and high microbial concentrations can be applied in the Integrated Laboratory of Poltekkes Kemenkes Denpasar.

B. Accuracy

Accuracy refers to the ability of a method to measure the true value or actual value of an analyte, such as the target microorganism (18). If the analyte is naturally present in the sample or intentionally added as part of a proficiency test, the method must be able to detect or recover the analyte at the correct concentration or frequency to be considered accurate. Accuracy is expressed as the percentage of analyte recovery from the added spike.

Table 3

Accuracy Test of Different Spike Colonies (SK)

NO	SK 10^{-2} CFU	LOG CFU	% recovery	SK 10^{-4} CFU	LOG CFU	% recovery	SK 10^{-7} CFU	LOG CFU	% recovery
1	70	1,84510	92,2549	15300	4,18469	104,6173	10600000	7,02531	100,3615
2	30	1,47712	73,85606	16500	4,21748	105,4371	12800000	7,10721	101,5316
3	90	1,95424	97,71213	16400	4,21484	105,3711	11500000	7,06070	100,8671
4	70	1,84510	92,2549	14800	4,17026	104,2565	13100000	7,11727	101,6753
5	50	1,69897	84,9485	15000	4,17609	104,4023	11100000	7,04532	100,6475
6	40	1,60206	80,103	16200	4,20952	105,2379	12500000	7,09691	101,3844
7	50	1,69897	84,9485	15800	4,19866	104,9664	11600000	7,06446	100,9208
8	60	1,77815	88,90756	10500	4,02119	100,5297	10500000	7,02119	100,3027
9	70	1,84510	92,2549	18300	4,26245	106,5613	11600000	7,06446	100,9208
10	30	1,47712	73,85606	16900	4,22789	105,6972	11800000	7,07188	101,0269
11	80	1,90309	95,1545	20300	4,30750	107,6874	10200000	7,00860	100,1229
12	60	1,77815	88,90756	20900	4,32015	108,0037	12500000	7,09691	101,3844
13	60	1,77815	88,90756	17400	4,24055	106,0137	12600000	7,10037	101,4339
14	30	1,47712	73,85606	15900	4,20140	105,0349	12800000	7,10721	101,5316
15	50	1,69897	84,9485	18000	4,25527	106,3818	12100000	7,08279	101,1826
16	60	1,77815	88,90756	11000	4,04139	101,0348	11500000	7,06070	100,8671
Rata-rata		1,72722	86,36114		4,20308	105,0771		7,07070	101,0101

Table 4
Accuracy (% Recovery) of TPC Testing in Goat Milk

No	**Dairy Goat Milk Sample**	**Average Log CFU**	**% Recovery Value**
1	Low microbial concentration (10^{-2} CFU)	1,72722	86,36114
2	Medium microbial concentration (10^{-4} CFU)	4,20308	105,0771
3	High microbial concentration (10^{-7} CFU)	7,07070	101,0101

Based on Tables 3 and 4, the percentage recovery values for TPC testing in dairy goat milk with low, medium, and high bacterial concentrations fall within the acceptable recovery percentage range (85–115%). Therefore, the TPC testing results using SNI 2897:2008 for dairy goat milk are considered accurate.

The verification of the TPC testing method in dairy goat milk is conducted to ensure that a method can be applied in the laboratory under real conditions through testing and proving that specific requirements have been met. Accuracy and precision determination should be performed with seven repetitions (10). However, in this study, eight repetitions were conducted to improve accuracy and minimize systematic errors during the analysis stage.

Conclusion

The Total Plate Count (TPC) testing method according to SNI 2897:2008, using a dairy goat milk matrix, can be applied as a testing method in the Integrated Laboratory of Poltekkes Kemenkes Denpasar.

Recommendations

Future researchers can conduct verification of the Total Plate Count (TPC) testing method according to SNI 2897:2008 using different matrices.

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