Vietnam Journal of Food Control
A Scientific Journal of National Institute for Food Control
Journal homepage: http://vjfc.vn/

Research Article

Evaluation of the bacterial detection capability of the LuciPac™ A3 Surface kit using the Lumitester Smart system for surface hygiene monitoring

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(Received: 24 Jan 2026; Revised: 21 May 2026; Accepted: 09 Jun 2026)

Abstract

Surface hygiene monitoring is essential for controlling microbial contamination in food processing environments, yet conventional culture-based methods are time-consuming and unsuitable for rapid on-site assessment. The LuciPac™ A3 Surface method combined with the Lumitester Smart system enables rapid detection of total adenylate (ATP, ADP, and AMP) as an indicator of biological contamination. This study evaluated the detection capability and repeatability of the A3 method in comparison with conventional microbiological methods using representative ATCC strains. The A3 method detected most microorganisms at approximately 10^5 CFU/mL, with lower detection limits observed for spore-forming bacteria, including *Bacillus cereus* (2.2 x 10^3 CFU/mL) and *Clostridium perfringens* (1.0 x 10^4 CFU/mL). Repeatability was acceptable, with relative standard deviation values ranging from 10% to 35%. Heat treatment increased RLU values, confirming the detection of total adenylate following cell disruption. Overall, the A3 method is suitable as a rapid screening tool for surface hygiene monitoring, although it does not replace conventional microbiological methods for confirmatory analysis.

Keywords: *LuciPac A3, ATP-ADP-AMP, surface hygiene monitoring, food safety, rapid method*.

1. INTRODUCTION

In the food production and processing chain, food-contact surfaces such as processing equipment, utensils, and workers' hands are recognized as potential sources of microbial contamination and cross-contamination. Inadequate surface hygiene has been identified as one of the major contributing factors to food poisoning and foodborne diseases, particularly in centralized food production facilities and communal kitchens [1]. Conventional microbiological methods based on culture and colony enumeration are still regarded as the gold standard for the identification and quantification of microorganisms. However, these methods require prolonged analysis times, typically ranging from 24 to 72 h, and therefore do not meet the requirements for rapid, on-site assessment of hygiene conditions, limiting their suitability for routine monitoring purposes.

In addition to culture-based methods, alternative analytical approaches have been developed, including nucleic acid-based techniques such as polymerase chain reaction (PCR) and real-time PCR (qPCR), as well as protein-based methods such as enzyme-linked immunosorbent assay (ELISA) [2]. These methods offer improved sensitivity and specificity; however, they require specialized equipment, trained personnel, and are not always suitable for rapid, on-site hygiene monitoring [3]. In practice, regulatory frameworks for food hygiene primarily rely on culture-based enumeration and indicator organisms, with defined limits expressed in CFU, while rapid methods such as ATP assays are typically used as supplementary tools for hygiene verification rather than direct compliance assessment.

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https://doi.org/10.47866/2615-9252/vjfc.4676

Rapid hygiene monitoring methods have been developed and widely applied, among which ATP-based bioluminescence assays are considered effective tools due to their simplicity, short analysis time, and suitability for field deployment. ATP is an energy-carrying molecule present in all living cells and various forms of biological matter; consequently, residual ATP detected on surfaces after cleaning is commonly used as an indirect indicator of the presence of organic and biological contamination [4, 5]. Despite these advantages, ATP-only detection methods exhibit inherent limitations. ATP can be degraded into adenosine diphosphate (ADP) and adenosine monophosphate (AMP) under the influence of enzymatic activity, temperature, pH, and chemical agents commonly used during cleaning and disinfection procedures [6]. This degradation may lead to an underestimation of residual biological contamination when ATP measurements are used alone, particularly following chemical treatment or incomplete cleaning processes [1, 5].

The LuciPac™ A3 Surface kit (Kikkoman Biochemifa) was developed to address this limitation by enabling the simultaneous detection of ATP, ADP, and AMP, thereby providing a more comprehensive assessment of organic and microbial contamination on surfaces [7]. This system is designed for rapid evaluation of microbial and organic residues on surfaces, in water, or in liquid samples and has been applied in various sectors, including food production, pharmaceuticals, healthcare, and environmental sanitation. The method is based on a bioluminescence reaction involving ATP, ADP, and AMP, collectively referred to as A3. In the presence of these nucleotides, a reaction occurs with luciferase and luciferin contained in the test device, producing light proportional to the total A3 content in the sample, which is measured by a luminometer and expressed as relative light units (RLU) [8]. The performance of this method has been validated under the AOAC Performance Tested Methods program. Previous studies have demonstrated acceptable linearity, repeatability, and sensitivity of the A3 method for detecting ATP, ADP, and AMP on stainless steel surfaces, as well as improved detection of biological residues compared with conventional ATP assays. However, the relationship between A3 results and microbiological indicators may vary depending on surface conditions and hygiene status [6, 7]. In Vietnam, systematic studies evaluating the detection capability of the A3 method across a wide range of pathogenic microorganisms and hygiene indicator organisms remain limited.

Based on these considerations, our study was conducted to evaluate the detection capability and applicability of the LuciPac™ A3 Surface kit in comparison with conventional microbiological methods for monitoring food-contact surface hygiene. The selected microorganisms represent groups commonly encountered in hygiene assessments, including Gram-negative bacteria, Gram-positive bacteria, and spore-forming bacteria. This selection reflects differences in microbial survival characteristics following cleaning and disinfection and is consistent with hygiene monitoring criteria commonly applied in the food industry.

2. MATERIALS AND METHODS

2.1. Bacterial strains

The microorganisms used in this study included *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Clostridium perfringens* ATCC 13124, and total aerobic microorganisms. These strains were selected as representative pathogenic and hygiene indicator microorganisms commonly encountered in food safety monitoring.

2.2. Reagents and equipment

2.2.1. Reagents

Brain heart infusion (BHI) broth (Merck, Germany), Butterfield phosphate buffer (Merck, Germany), and other reagents were used in accordance with the relevant reference methods and standard analytical procedures.

2.2.2. Equipments

The main equipment used in this study included Lumitester Smart systems (Serial Nos. 2209053130064S, 2209093130063S, and 220909313006S), a Memmert IN110 incubator, an Esco AC2 biological safety cabinet, multichannel pipettes with appropriate volume ranges, stainless steel plates (SUS304), Prowipe S220 wipes, micropipettes (2 - 20 µL, 20 - 200 µL, and 100 - 1000 µL), and other standard laboratory accessories.

2.3. Research methodology

2.3.1. A3 bioluminescence method

2.3.1.1. Preparation of the dilution solution

The dilution solution was prepared by reconstituting Butterfield phosphate buffer from KH_2PO_4 at pH 7.0. The prepared buffer was aliquoted into 9 mL glass tubes and sterilized at 121°C for 15 min. The sterile phosphate buffer was subsequently used for bacterial dilution to maintain cell stability and minimize the influence of external factors on experimental results.

2.3.1.2. Preparation of bacterial suspensions

The bacterial strains were cultured in BHI broth at 37°C for 24 h. Following incubation, 1 mL of each culture was centrifuged at 10,000 rpm for 1 min to collect the cell pellet. The pellet was washed twice with phosphate buffer, with each washing step followed by centrifugation at 10,000 rpm for 1 min to remove residual culture medium. The final cell pellet was resuspended in 1 mL of phosphate buffer to obtain the stock bacterial suspension for subsequent experiments.

2.3.1.3. Surface inoculation and A3 measurement

Experiments were performed on stainless steel surfaces (SUS304) with dimensions of 50 x 50 cm. The surfaces were sterilized at 121°C for 15 min and wiped with Prowipe paper inside a biological safety cabinet prior to use. Stock bacterial suspensions were serially diluted to obtain a range of contamination levels. These dilutions were used to evaluate the response of the A3 method across predefined operational RLU ranges (1000 - 500, 500 - 200, 200 - 75, and < 75 RLU), which represent commonly applied hygiene interpretation thresholds rather than values established through calibration. At each dilution level, 250 µL of bacterial suspension was evenly spread onto the surface and allowed to dry at room temperature. Surfaces were then swabbed using the LuciPac™ A3 Surface kit according to the manufacturer's instructions, and RLU values were measured using the Lumitester Smart system [9]. Each condition was tested in ten replicates. In this study, the A3 method was applied as a rapid biochemical screening tool, while conventional microbiological methods were used as the reference for quantifying viable microorganisms. To assess the detection mechanism, untreated samples were compared with heat-treated samples (100°C for 10 min), with five replicates per condition. Stainless steel (SUS304) was selected as a representative food-contact material due to its widespread use in food processing environments and its common application in hygiene validation studies.

2.3.2. Conventional microbiological methods

Microbial enumeration was performed using standard culture-based methods in accordance with Vietnamese national standards, including TCVN 4992:2005 for *B. cereus* [10], TCVN 4830-1:2005 for *S. aureus* [11], TCVN 7924-1:2019 for *E. coli* [12], TCVN 4884-1:2015 for total aerobic microorganisms [14], TCVN 8881:2011 for *P. aeruginosa* [14], and TCVN 4991:2005 for *C. perfringens* [15]. All procedures followed standard protocols for sample preparation, dilution, inoculation, incubation, and enumeration.

2.3.3. Statistical analysis

Experimental data were processed using Microsoft Excel. Results are presented as mean ± standard deviation (Mean ± SD). Relative standard deviation (RSD, %) was calculated as (SD/Mean) x 100. Statistical differences between groups were evaluated using one-way ANOVA ($p < 0.05$). Heat treatment effects were analyzed using two-way ANOVA. Where appropriate, two-sample t-tests with multiple comparison correction were applied. Graphs were generated using GraphPad Prism software (GraphPad Software, USA).

3. RESULTS AND DISCUSSION

3.1. Results of bacterial quantification using traditional microbiological methods

Quantification using conventional microbiological methods demonstrated that all tested bacterial strains exhibited stable growth under standard culture conditions, reaching cell densities suitable for establishing dilution levels for evaluating the detection threshold of the A3 method (**Table 1**). *E. coli* reached a concentration of 6.4 x 10^8 CFU/mL, *S. aureus* 1.0 x 10^9 CFU/mL, *B. cereus* 4.0 x 10^6 CFU/mL, *P. aeruginosa* 1.0 x 10^8 CFU/mL, *C. perfringens* 7.6 x 10^7 CFU/mL, and total aerobic microorganisms 2.0 x 10^8 CFU/mL. The relative standard deviation (RSD) values for all tested microorganisms were approximately 5%, indicating good repeatability and analytical stability of the conventional enumeration procedures. These results confirm the reliability of the initial bacterial concentrations used as reference values for subsequent dilution and comparative analysis with the A3 method. Based on the mean concentrations obtained, appropriate dilution

series were prepared to generate bacterial levels corresponding to the target RLU ranges evaluated in the A3 experiments.

***Table 1**. Results of bacterial quantification using traditional microbiological methods*

Bacteria	Test method	Bacterial concentration (CFU/mL)			Mean concentration (CFU/mL)	SD	RSD (%)
		1	2	3			
E. coli ATCC 25922	TCVN 7924-1:2019	6.1×10^8	6.4×10^8	6.7×10^8	6.4×10^8	3.2×10^7	5.0
S. aureus ATCC 25923	TCVN 4830-1:2005	9.5×10^8	1.0×10^9	1.1×10^9	1.0×10^9	5.0×10^7	5.0
B. cereus ATCC 10876	TCVN 4992:2005	3.8×10^6	4.0×10^6	4.2×10^6	4.0×10^6	2.0×10^5	5.0
P. aeruginosa ATCC 27853	TCVN 8881:2011	9.5×10^7	1.0×10^8	1.1×10^8	1.0×10^8	5.0×10^6	5.0
C. perfringens ATCC 13124	TCVN 4991:2005	7.2×10^7	7.6×10^7	8.0×10^7	7.6×10^7	3.8×10^6	5.0
Total aerobic microorganisms	TCVN 4884-1:2015	1.9×10^8	2.0×10^8	2.1×10^8	2.0×10^8	1.0×10^7	5.0

3.2. A3 response across bacterial concentrations

The results presented in **Table 2, Table 3, Table 4** and **Figure 1** show the response of the A3 method across different bacterial concentrations. For all tested microorganisms, RLU values decreased progressively with decreasing bacterial loads, indicating a concentration-dependent response of the A3 assay. At concentrations of approximately 10^5 CFU/mL, RLU values were significantly higher than those of the blank sample (phosphate buffer), as determined by one-way ANOVA ($p < 0.05$), demonstrating the ability of the method to discriminate contaminated from clean surfaces.

The A3 method provides results within approximately 30–60 s per sample, excluding sample preparation time, which is significantly faster than conventional culture-based methods requiring 24–72 h. This rapid response enables timely hygiene assessment and supports its application as a screening tool in food processing environments.

3.3. Comparison of results obtained from method A3 and traditional microbiological methods

The results obtained from method A3 and traditional microbiological methods were showed in **Table 2, Table 3** and **Table 4**. To further evaluate the performance of the A3 method, RLU values were compared with bacterial concentrations determined by conventional microbiological methods. For most bacterial groups, RLU values obtained at concentrations of approximately 10^5 CFU/mL were significantly different from those of the blank sample ($p < 0.05$), including *E. coli*, *S. aureus*, *P. aeruginosa*, and total aerobic microorganisms. Notably, the A3 method exhibited higher sensitivity for spore-forming bacteria. Statistically significant differences in RLU values ($p < 0.05$) were observed for *B. cereus* at a concentration of 2.2×10^3 CFU/mL and for *C. perfringens* at 1.0×10^4 CFU/mL. This enhanced sensitivity may be attributed to the higher stability of adenine nucleotides in spore-forming organisms and their release upon partial cellular disruption during sample handling. Across all tested microorganisms and concentration levels, most RSD values ranged from 10% to 35%, indicating acceptable repeatability for hygiene monitoring applications. This level of variability is comparable to that reported in food-based validation studies and supports the suitability of the A3 method as a screening tool for surface hygiene assessment. The observed detection thresholds and repeatability are consistent with previous studies conducted in Japan on *Cronobacter sakazakii, Lactobacillus acidophilus, and Saccharomyces cerevisiae* [9]. In those studies, the detection limit of the A3 method for foodborne pathogens such as *C. sakazakii* was approximately 1.5×10^5 CFU/mL, while for anaerobic spore-forming bacteria such as *L. acidophilus*, the detection limit was reported at 1.0×10^4 CFU/mL. These findings align closely with the results obtained for *C. perfringens* in the present study. Overall, the comparative analysis demonstrates that

the A3 method is capable of detecting a broad range of pathogenic and hygiene indicator microorganisms, with detection performance varying according to microbial type and physiological characteristics.

***Table 2**. Comparison of results obtained from method A3 and traditional microbiological methods for E. coli and S. aureus*

Bacteria		***E. coli***					***S. aureus***				
Target RLU value		1000-500	500-200	75-200	< 75	Blank	1000-500	500-200	75-200	< 75	Blank
Bacterial concentration determined by traditional methods (CFU/mL)		5.0 x 10^7	3.5 x 10^6	1.2 x 10^5	1.0 x 10^4	0	1.4 x 10^7	1.1 x 10^6	1.0 x 10^5	1.0 x 10^4	0
Actual RLU values obtained from method A3	1	1013	293	84	58	37	1106	199	80	55	35
	2	783	322	103	58	38	1030	178	86	43	36
	3	1058	400	90	34	36	1174	186	88	55	38
	4	835	422	101	42	42	1253	198	88	43	42
	5	850	311	64	35	39	860	261	48	48	44
	6	875	200	69	25	40	932	267	76	42	40
	7	968	335	80	36	42	950	185	125	38	40
	8	1346	201	95	39	38	1000	180	103	38	42
	9	1212	272	87	35	40	1125	283	75	40	51
	10	1061	216	77	40	51	1156	213	75	37	39
Average RLU value		1000	297	85	40	40	1059	215	84	44	41
Standard deviation		178	78	13	10	4	124	40	20	7	5
RSD (%)		17.8	26.1	15.2	26	10.5	11.7	18.6	23.7	15.2	11.2

***Table 3**. Comparison of results obtained from method A3 and traditional microbiological methods for B. cereus and P. aeruginosa*

Bacteria		***B. cereus***					***P. aeruginosa***				
Target RLU value		1000-500	500-200	75-200	< 75	Blank	1000-500	500-200	75-200	< 75	Blank
Bacterial concentration determined by traditional methods (CFU/mL)		1.2 x 10^5	4.0 x 10^4	2.2 x 10^3	2.0 x 10^2	0	1.0 x 10^7	2.0 x 10^6	1.5 x 10^5	1.0 x 10^4	0
Actual RLU values obtained from method A3	1	960	465	69	15	10	1143	260	112	43	29
	2	1035	379	74	18	8	1147	266	76	46	37
	3	1076	415	70	8	9	570	220	60	48	36
	4	1009	503	71	8	9	1426	226	106	61	50
	5	980	333	124	17	7	452	246	73	39	52
	6	1062	318	120	14	10	983	252	99	66	38
	7	852	520	120	7	9	982	201	94	62	40
	8	1117	479	132	7	8	884	285	145	66	40
	9	931	354	164	8	6	1029	339	111	42	42
	10	602	367	160	9	6	913	285	103	40	41
Average RLU value		962	413	110	11	8	953	258	98	51	41
Standard deviation		148	74	37	4	1	281	39	24	11	7
RSD (%)		15.4	17.8	33.6	39.5	18	29.5	15.3	24.6	21.7	16.4

***Table 4**. Comparison of results obtained from method A3 and traditional microbiological methods for C. perfringens and total aerobic microorganisms*

Bacteria		***C. perfringens***					**Total aerobic microorganisms**				
Target RLU value		1000 - 500	500 - 200	75 - 200	< 75	Blank	1000 - 500	500 - 200	75 - 200	< 75	Blank
Bacterial concentration determined by traditional methods (CFU/mL)		1.0 x 10^6	2.1 x 10^5	1.0 x 10^4	1.1 x 10^3	0	1.4 x 10^7	1.1 x 10^6	2.0 x 105	1.0 x 10^4	0
Actual RLU values obtained from method A3	1	946	290	84	21	10	656	162	96	46	26
	2	1128	281	124	21	13	798	252	86	40	16
	3	1226	305	129	19	11	556	166	90	45	20
	4	981	298	90	20	13	641	259	96	32	24
	5	1016	305	71	12	20	506	225	86	34	16
	6	1272	565	97	15	15	503	210	80	28	24
	7	969	379	75	11	12	578	218	83	30	20
	8	758	415	76	11	17	551	231	81	34	22
	9	825	483	80	15	8	579	217	77	37	28
	10	1023	400	72	9	9	580	230	82	27	14
Average RLU value		1014	372	90	15	13	595	217	86	35	21
Standard deviation		161	95	21	5	4	87	32	6	7	5
RSD (%)		15.9	25.6	23.4	29.7	29	14.6	14.7	7.6	18.8	22.1

The dependence of RLU value on concentration as bacterial load decreases is shown in **Figure 1**.

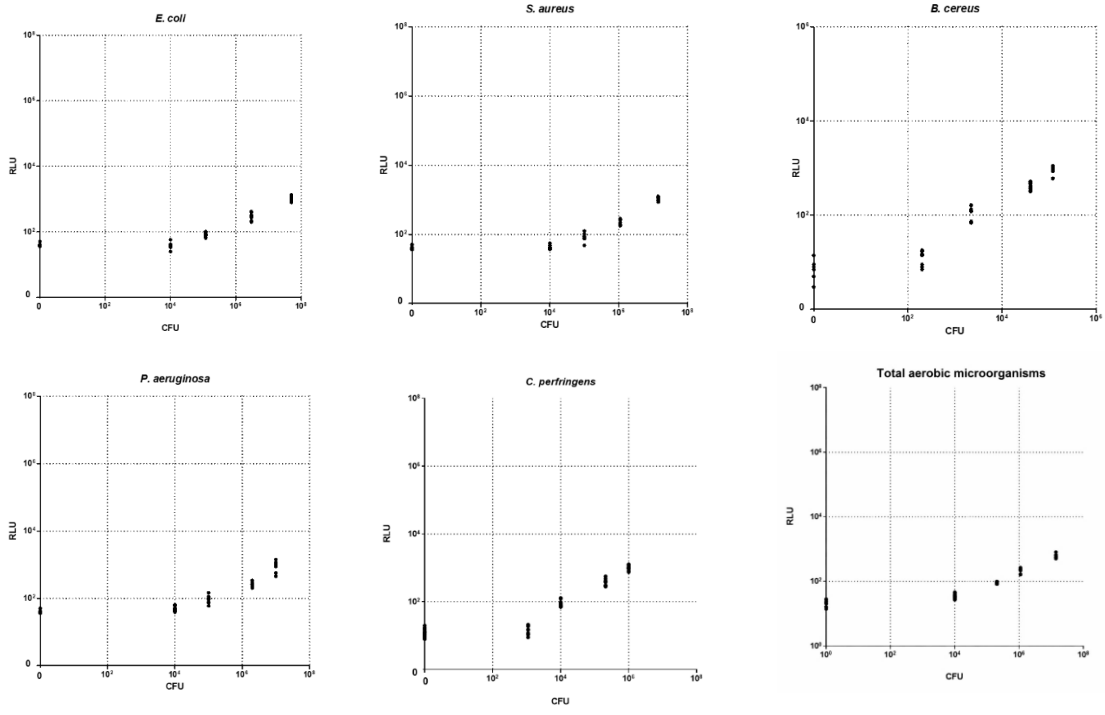

***Figure 1**. Chart comparing results obtained from method A3 and traditional microbiological methods.*

Figure 1 demonstrates a concentration-dependent decrease in RLU values with decreasing bacterial loads, indicating that the A3 method assay is responsive to changes in surface microbial contamination. For most tested organisms, signals at approximately 10^5 CFU/mL were statistically distinguishable from the blank ($p <$

0.05), confirming the method's ability to discriminate contaminated from clean surfaces. Species-specific response patterns were observed, particularly for spore-forming bacteria, which showed detectable differences at lower CFU levels, reflecting the measurement of total adenylate nucleotides rather than viable counts alone. These results support the use of the A3 assay as a rapid hygiene screening tool, while highlighting that RLU values should not be directly interpreted as CFU without confirmatory microbiological analysis.

3.3. Results of the effect of heat treatment on RLU values

The results presented in **Table 5** and **Figure 2** show that heat treatment resulted in a marked increase in RLU values for total aerobic microorganisms compared with untreated samples at concentrations ranging from 10^8 to 10^5 CFU/mL. Two-way ANOVA confirmed that heat treatment had a statistically significant effect on the RLU signal ($p < 0.05$), and a significant interaction was observed between bacterial concentration and treatment condition. The observed increase in RLU values following heat treatment can be explained by the disruption of microbial cell membranes and the inactivation of intracellular enzymatic systems, leading to the release of intracellular adenine nucleotides into the surrounding environment. As the A3 method simultaneously detects all three nucleotide forms, the resulting bioluminescence signal more accurately reflects the total amount of biological material present on the surface. In contrast, at lower concentrations (10^4 CFU/mL) and in blank samples, no statistically significant differences were observed between untreated and heat-treated conditions. This indicates that the amount of released nucleotides did not exceed the baseline detection level of the method under the experimental conditions, thereby reflecting the practical detection limit of the A3 system.

***Table 5**.Heat treatment testing for total aerobic microorganisms.*

Concentration (CFU/mL)	Processing conditions	The RLU value obtained					Average RLU value
		1	2	3	4	5	
10^8	Normal	8315	7090	8253	8831	7952	8088 ± 640
	Heat treatment	99207	107483	114018	122234	112914	114571 ± 12047
10^7	Normal	656	798	556	641	824	695 ± 111
	Heat treatment	10123	10053	11953	11028	11573	10946 ± 806
10^6	Normal	162	252	166	259	202	208 ± 45
	Heat treatment	868	922	870	838	901	880 ± 34
10^5	Normal	48	43	45	48	50	47 ± 3
	Heat treatment	129	120	125	116	120	122 ± 5
10^4	Normal	10	14	14	14	17	14 ± 2
	Heat treatment	10	13	11	16	19	14 ± 4
Blank	Normal	5	7	5	9	10	7 ± 2
	Heat treatment	10	9	1	5	4	6 ± 3

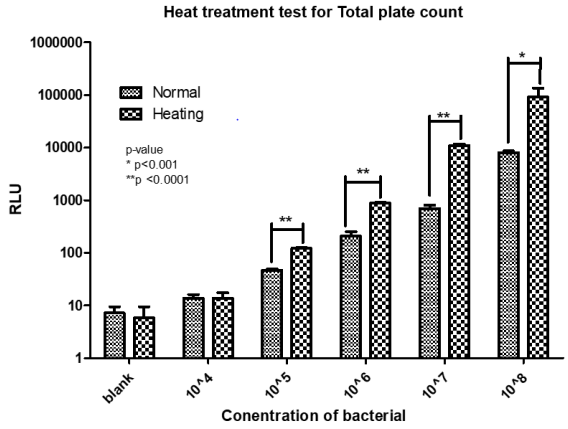

***Figure 2**. Heat treatment test curve for total aerobic microorganisms.*

These findings suggest that the A3 method is particularly suitable for rapid surface hygiene monitoring and for assessing overall levels of biological contamination. However, because the method does not differentiate between viable and non-viable microorganisms, A3 results should be interpreted with caution and, where necessary, complemented by conventional microbiological methods when the objective is to assess the presence of live microorganisms or to comply with CFU-based regulatory criteria.

4. CONCLUSION

The LuciPac™ A3 Surface kit used with the Lumitester Smart system was able to detect biological contamination from a broad range of tested microorganisms and showed acceptable repeatability for rapid hygiene screening. For most organisms, signals above the blank were obtained at approximately 10^5 CFU/mL, whereas lower apparent detection limits were observed for *B. cereus* and *C. perfringens*. Overall, the A3 method is suitable as a rapid screening tool for surface hygiene monitoring.

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