Original Article

Correlation among monitoring methods of surface cleaning and disinfection in outpatient facilities

Correlação entre métodos de monitoramento de limpeza e desinfecção de superfícies ambulatoriais Correlación entre métodos de monitoreo de limpieza y desinfección de superficies en unidades ambulatorias

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Keywords

Adenosine triphosphate; Monitoring; Housekeeping; Disinfection, Ambulatory care

Descritores

Trifosfato de adenosina; Monitoramento; Servico de limpeza; Desinfecção; Assistência ambulatorial

Descriptores

Adenosina trifosfato; Monitoreo fetal; Servicio de limpieza; Desinfección; Atención ambulatoria

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Abstract

Objectives: To evaluate the correlation among microbiological culture, ATP bioluminescence assay, and visual inspection in monitoring the effectiveness of surface cleaning and disinfection in an outpatient facility and determine the ATP bioluminescence cutoff capable of indicating a

Clean surface regarding microbiological evaluation. Methods: Exploratory, cross-sectional, and correlation study consisting of 720 evaluations in five surfaces before and after cleaning and disinfection. The results were used to run two-proportions tests, calculate Spearman's correlation, and plot the receiver operating characteristic

Results: Similar proportions (p>0.05) occurred for non-approval rates between ATP-bioluminescence and aerobic colony count only when the evaluations of all the surfaces before and after cleaning and disinfection were put together. There was a significant correlation between the ATP quantification and microbial count methods for the reception desk and the stretcher. Receiver operating characteristic analysis indicated that ATP quantification showed a significant result in comparison with aerobic colony count (p=0.044)

conclusion: There was a discrete correlation between the ATP quantification and microbial count methods for two surfaces. It is suggested that surfaces showing values ≤49 relative light units are clean.

Resumo

Objetivo: Avaliar a correlação entre cultura microbiológica, teste de ATP por bioluminescência e inspeção visual na monitorização da eficiência da limpeza e da desinfecção de superfícies de uma unidade ambulatorial e determinar o valor de corte de ATP-bioluminescência capaz de indicar superfície limpa em relação à avaliação microbiológica.

Métodos: Estudo exploratório, longitudinal e correlacional. Foram realizadas 720 avaliações em cinco superfícies antes e após a limpeza e a

desinfecção. Nos resultados, foram realizadas análises de duas proporções, a correlação de *Spearman* e a curva ROC. Resultados: Ocorreram proporções semelhantes (p≥0,05) entre as taxas de reprovação apenas entre ATP-bioluminescência e contagem de colônias aeróbias (CCA) quando somadas as avaliações de todas as superfícies antes e depois da limpeza e da desinfecção. Houve correlação significativa entre os métodos de quantificação de ATP e a contagem microbiana para o balção da recepção e a maca. A análise ROC indicou que a quantificação de ATP apresentou resultado significativo na comparação com a CCA (p=0,044). Conclusão: Embora discreta, houve correlação significativa entre os métodos de quantificação de ATP e contagem microbiana para duas

superfícies. Sugere-se que superfícies que apresentam valores ≤49 unidades relativas de luz estão limpas.

Resumen

Objetivo: Analizar la correlación entre cultivo microbiológico, prueba de ATP por bioluminiscencia e inspección visual en el monitoreo de la eficiencia de la limpieza y desinfección de superficies en una unidad ambulatoria y determinar el valor de referencia de ATP-bioluminiscencia que indique que la superficie está limpia con relación a la evaluación microbiológica. Métodos: Estudio exploratorio, longitudinal y correlacional. Se realizaron 720 evaluaciones en cinco superficies antes y después de la limpieza y

desinfección. En los resultados, se realizaron análisis de dos proporciones: la correlación de Spearman y la curva ROC

Resultados: Hubo proporciones semejantes (p≥0,05) entre los índices de reprobación solo entre ATP-bioluminiscencia y recuento de colonias aerobias (RCA) cuando se sumaron las evaluaciones de todas las superficies antes y después de la limpieza y desinfección. Hubo una correlación significativa entre los métodos de cuantificación de ATP y el recuento microbiano en el mostrador de la recepción y la camilla. El análisis ROC indicó que la cuantificación de ATP presentó un resultado significativo en la comparación con el RCA (p=0,044).

Conclusión: Hubo una correlación significativa, aunque discreta, entre los métodos de cuantificación de ATP y recuento microbiano en dos superficies. Se sugiere que las superficies que presentan valores ≤49 unidades relativas de luz están limpias.

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³Instituto de Higiene e Medicina Tropical, Universidade NOVA de Lisboa, Lisboa, Portugal. Conflicts of interest: Originated from the doctoral thesis "Limpeza e desinfecção de superfícies em uma clínica de cuidados especializados: impacto de um programa de intervenções", by Mara Cristina Ribeiro Furlan, advised by Prof. Adriano Menis Ferreira and presented to the Graduate Program in Health and Development in the Center-West Region of the Federal University of Mato Grosso do Sul in June 2018 as a prerequisite to obtain a PhD title.

Introduction

The definition of healthcare-associated infections (HAIs) has come up to meet the need to evaluate infections in non-hospital settings, given that patients receiving care in several facilities that go beyond the hospital setting are an increasingly more common reality. However, this initiative has not eliminated the protagonism that hospital settings have in the literature, because most studies on HAIs are carried out in hospitals.⁽¹⁾

As a consequence, evidence to guide care in basic health units (BHUs), outpatient facilities, and emergency care units on good practices is scarce. This statement is even more accurate when cleaning and disinfection of surfaces are considered.⁽²⁾

Many microorganisms are present on surfaces that are highly touched and close to patients, such as desks, tables, and stretchers.^(3,4) Although these surfaces are not considered critical, because they get in touch with patients' intact skin and not mucosas, they contribute to cross infection.⁽⁵⁾ From 30% to 60% of the surfaces close to patients colonized or infected by *Clostridium difficile*, vancomycin-resistant enterococci, or methicillin-resistant *Staphylococcus aureus* are also contaminated with these microorganisms.⁽⁶⁻⁸⁾ In addition, studies point out that contamination of environmental surfaces increases by 120% the possibility of susceptible patients occupying a contaminated room to be colonized or infected by these microorganisms.⁽⁶⁻⁸⁾

Pathogenic agents can survive in environmental surfaces for days, weeks, and even months.⁽⁹⁾ Nevertheless, cleaning and disinfection reduce the level and frequency of contamination and the risk of HAIs considerably if the practices are carried out correctly.⁽⁹⁾ Despite this positive outcome, routine cleaning and disinfection practices are usually performed incorrectly.⁽¹⁰⁾ Taking into account this perspective, an increasingly higher number of methods for evaluating cleaning and disinfection are being considered as part of infection prevention and control programs.⁽²⁾ Among these methods, visual inspection, microbiological evaluation, and ATP bioluminescence assay are the most commonly known and used.^(2,10-13) Visual inspection is the most commonly used evaluation method, and quite often the only one. Despite assessing the esthetic aspect and having a low cost, this method does not evaluate the microbiological risk and consequently does not provide quantitative feedback on the effectiveness of sanitization in the cleaning and disinfection process.⁽¹²⁾

The ATP bioluminescence assay gives healthcare teams immediate feedback and is easy to use. However, its disadvantages are low sensitivity and specificity, relatively high cost, and constant technological changes, which makes the cutoff value to determine surface cleaning and disinfection different depending on the technology applied and hinders comparisons among studies.⁽¹⁴⁾

The microbiological culture evaluation method is considered the gold standard to detect microorganisms, but it does not provide immediate feedback, given that it takes from 24 to 48 hours for microorganisms to grow. Additionally, it requires greater financial resources and an available laboratory.⁽¹⁵⁾

Each monitoring method has positive and negative points. Therefore, their combined application is preferable to their use in isolation.⁽¹³⁻¹⁷⁾ Consequently, it is fundamental to correlate the available monitoring methods, especially in outpatient settings, for which studies are scarce, to obtain evidence to implement good practices for prevention and control of HAIs.

The main objective of the present study was to evaluate the correlation among microbiological culture, ATP bioluminescence assay, and visual inspection in monitoring the effectiveness of surface cleaning and disinfection in an outpatient facility. The secondary objective was to determine an ATP bioluminescence cutoff value that can indicate whether a surface is clean.

Methods

Study design, setting, and period

The present study is analytical, comparative, and had a prospective collection. It was carried out in July, September, and December 2015 in an outpatient clinic that offers services of medical specialties, outpatient surgeries, and treatment for chronic injuries to a population of over 100,000 people in the interior of the state of Mato Grosso do Sul, Brazil.

Institution standard protocol

The cleaning and disinfection of the examined surfaces were executed by nursing and cleaning teams. The reception desk was assigned to the latter and the other surfaces to the former. The cleaning and disinfection of the stretcher at the dressing room were carried out at the end of the procedures applied to each patient, and the other surfaces were hygienized at the end of each shift (morning and afternoon). The product used to clean the surfaces was made up of 12.4% glucoprotamin and 15% alkyl-dimethyl-benzyl-ammonium chloride (Ecolab Deutschland GmbH, Düsseldorf, Germany).⁽¹⁸⁾ This product has detergent and disinfectant functions, and therefore cleans and disinfects in a single step.

Study protocol

Five environmental surfaces with a higher frequency of touch and that were closer to patients and professionals were selected for the sample, according to the directions of the literature.⁽¹⁸⁾ By using purposive non-probability sampling, surfaces at the dressing room (dressing trolley and stretcher), reception (reception desk), and outpatient surgery room (support table and operating table) were chosen. The surfaces were selected based on systematic observation and indication of the nurses that provide care to patients at the facility. Only the outpatient surgery room had electronic equipment, and it showed a lower frequency of use than the other chosen surfaces.

Ten samples were collected from the five examined surfaces, five before and five after the cleaning and disinfection process, twice a week. The monitoring methods used to assess the cleaning and disinfection of the surfaces were visual inspection, ATP bioluminescence assay, and aerobic colony count (ACC). The surfaces were sampled exclusively by a researcher immediately before and ten minutes after completion of the morning and afternoon cleaning and disinfection session, depending on the surface that was going to be used in the period. This procedure allowed the surfaces to dry up and consequently prevented the contact between sanitizing products and reagents from influencing relative light units (RLUs) and ACC values.⁽¹⁹⁾

Adopted concepts and parameters

In the visual inspection method, a surface was considered dirty when it showed the presence of at least one of the following items: dust, liquids, detritus (organic matter or not), ink stains, and glue.⁽¹⁹⁾

The ATP bioluminescence assay was applied to measure the quantity of organic matter by using a portable luminometer (NGi 3M[™] Clean-Trace[™], St. Paul, MN) and a swab (3M[™] Clean-Trace[™] ATP Surface). Collection was carried out in line with the directions of the manufacturer, according to which a pre-dampened swab must be scrubbed in an area of 100 cm², first covering the area with a back and forth template, and then with an overlaid back and forth template, perpendicular to the first one, executing a twist movement so the entire swab is exposed to the surface. The samples were analyzed immediately after collection. The amount of ATP in the samples was quantified as RLUs. The surfaces were classified as clean when the reading in the equipment was lower than 250 RLUs.^(15,20-21)

To monitor total aerobic microorganisms, Rodac plates^{*} (Biocen do Brasil) with a contact area of 24 cm² and made up of tryptone soy agar and neutralizing agents were used. The plates were pressed for ten seconds at a place adjacent to that where samples for ATP bioluminescence analysis were obtained on the examined surfaces. Subsequently, the plates were inserted in an incubator at 37 °C and kept there for a period between 24 and 48 hours.⁽²²⁾

Plate count was carried out using a digital colony counter (Logen LS6000; Texas Instruments Inc., Dallas, TX). Surfaces were considered clean if they had a count lower than 2.5 CFU/cm², that is, less than 60 colony-forming units in a 24 cm² plate.⁽¹⁸⁾

Statistical analysis

Data were analyzed using the following statistical tests: two-proportions test, to compare the frequency of non-approved surfaces among the monitoring methods (visual inspection, ATP bioluminescence, and ACC); Spearman's correlation, to detect possible correlations among the quantifications of continuous variables (ATP bioluminescence and microbial count on each surface, before and after cleaning and disinfection); and the receiver operating characteristic (ROC) curve, to verify whether the ATP bioluminescence assay is effective to determine the quality of surface cleaning and disinfection in comparison with the microbiological evaluation gold standard. All the statistical tests were applied with a 5% level of significance or p<0.05 and the software used to run the analyses were Minitab 17 (Minitab Inc.) and MedCalc 16.8 (MedCalc^{*}).

Ethical procedures

The present study met all national and international ethical principles and was approved by the Human Research Ethics Committee of the Federal University of Mato Grosso do Sul as per report 1006802/2015.

Results

Of all 720 samples collected using the three monitoring methods (visual inspection, ATP bioluminescence, and ACC), half was collected before and half after cleaning and disinfection. Each of the five surfaces was sampled 48 times in each monitoring method, totaling 240 evaluations per method. Among the 120 evaluations performed before cleaning and disinfection, 54.1%, 49.1%, and 45% were considered dirty according to visual inspection, ACC, and ATP bioluminescence, respectively, versus 45.8%, 12.5%, and 16.6% after cleaning and disinfection (Table 1).

In the two-proportions test, the value p<0.05 indicates a statistically significant difference, that is, when p is higher than 0.05, there is similarity among the non-approval rates of the examined monitoring methods. When the rates of the cluster of all the dirty surfaces (Table 1) were compared, the frequency of occurrence was similar (p>0.05) between ATP and visual inspection (p=0.518), ATP and ACC (p=0.605), and visual inspection and ACC (p=0.197) before cleaning and disinfection of the surfaces. After cleaning and disinfection, the similarity was observed only in the comparison between the results of ATP and ACC (p=0.465).

For analysis of surfaces before cleaning and disinfection, six specific cases of significantly different proportions were observed (p<0.05): three for the comparison between ATP and visual inspection and three for the comparison between ACC and visual inspection. In the comparison between ATP and visual inspection, the reception desk showed a higher level of dirt in the ATP bioluminescence method (50%), whereas the dressing trolley and the support table had the highest percentages of dirt according to visual inspection (100% for both objects). The comparison between ACC and visual inspection showed comparable results. The reception desk

Table '	1. Surface	type an	d monitoring	method f	for samples	collected before	and after	cleaning and	disinfection.
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	Visual	ΔTP (BI	lls/cm ²)	ACC (CEU/cm ²)		n valuet		
Time/surface	Surface presenting visible dirt n(%)	Median (variation)	Above the cutoff (<250 RLUs/cm ²) n(%)	Median (variation)	Above the cutoff (<2.5 CFU/cm ²) n(%)	ATP vs visual	ATP vs ACC	ACC vs visual
Before cleaning and disinfection								
Reception desk	-	273(71;1,365)	12(50)	56(7;300)	11(45.8)	< 0.001	1.000	< 0.001
Dressing trolley	24(100)	126.5(53;533)	8(33.3)	40(4;300)	8(33.3)	< 0.001	1.000	< 0.001
Stretcher	9(37.5)	322(59;3,809)	16(66.6)	68(3;257)	15(62.5)	0.082	1.000	0.148
Operating table	9(37.5)	413(50;9,597)	14(58.3)	27(2;300)	8(33.3)	0.248	0.147	1.000
Support table	24(100)	223(23;1,989)	9(37.5)	59.5(0;173)	12(50)	< 0.001	0.561	< 0.001
All	66(54.1)	250(23;9,597)	59(49.1)	47(0;300)	54(45)	0.518	0.605	0.197
After cleaning and disinfection								
Reception desk	-	128(16;792)	6(25)	17(2;153)	5(20.8)	0.022	1.000	0.050
Dressing trolley	24(100)	45(13;338)	1(4.2)	9.5(0;94)	3(12.5)	< 0.001	0.609	< 0.001
Stretcher	4(16.6)	76.5(22;2,083)	4(16.6)	6(0;176)	3(12.5)		1.000	1.000
Operating table	4(16.6)	90(11;1,415)	4(16.6)	3(0;178)	5(20.8)	1.000	1.000	1.000
Support table	24(100)	44(11;91)	0(0.0)	3(0;172)	4(16.6)	-	0.109	< 0.001
All	56(45.8)	59(11;2,083)	15(12.5)	9.5(0;178)	20(16.6)	< 0.001	0.465	<0.001

† Two-proportions test with p<0.05; the value p<0.05 indicates a statistically significant difference

showed a higher percentage of dirt in the microbial count method (45.8%), whereas the dressing trolley and the support table had higher levels of dirt in the visual inspection (Table 1).

Four specific cases of significantly different proportions were observed after cleaning and disinfection: two for the comparison between ATP and visual inspection and two for the comparison between ACC and visual inspection. For the first pair of monitoring methods, the reception desk showed a higher non-approval rate according to the ATP bioluminescence method (25%), whereas for the trolley the percentage of non-approval was higher in the visual inspection. For the second pair of monitoring methods, both cases (dressing trolley and support table) showed a higher percentage of non-approval in the visual inspection method (100% in both cases) (Table 1).

The Spearman's coefficient calculated for each surface individually indicated the presence of a significant correlation between the quantification provided by the ATP and ACC methods for the reception desk (rho=0.598; p=0.002) and the stretcher (rho=0.422; p=0.040) (Table 2).

Table 2. Evaluations of each surface before and after cleaning and disinfection according to the ATP-bioluminescence and microbial count monitoring methods

Surfaces	Spearman's coefficient	p value
Reception desk	0.598	0.002
Dressing trolley	0.141	0.512
Stretcher	0.422	0.040
Operating table	0.149	0.487
Support table	0.051	0.811

Analysis of the correlation between ATP quantification and ACC for the reception desk and the stretcher revealed a discrete, linear, and positive correlation (Figure 1), that is, the higher the ATP quantification, the higher the microbial count in these surfaces.

The evaluation of the ATP quantification method using bioluminescence in comparison with microbial count showed the following results: sensitivity of 53.3%; specificity of 66.7%; positive predictive value of 61.54%; and negative predictive value of 58.81%. Adopting the ACC reference of <2.5 CFU/cm² as the definition of a clean surface (reference method), ROC analysis indicates that surfaces



Figure 1. Correlation between ATP quantification and microbial count for reception desk and stretcher

with ATP results lower than 49 RLUs (Figure 2) can be considered approved, this being the point of highest specificity and sensitivity.



Figure 2. ROC curve of the ATP bioluminescence quantification method compared with the gold standard, represented by microbial count

Discussion

The findings showed that, in general, there were no similar proportions of non-approval of the surfaces between visual inspection and ATP bioluminescence (p<0.001) or visual inspection and ACC (p<0.001) after cleaning and disinfection (Table 1).

These results may have been influenced by the state of conservation of the examined surfaces. All the surfaces that were considered dirty according to visual inspection, both before and after cleaning and disinfection, had grooves and peeling of the paint. Consequently, even if the cleaning and disinfection procedure is executed correctly, it would be necessary to change or repair these surfaces for possible approval in the future.

A study carried out in Scotland corroborates the findings of the present study: visual inspection did not show a correlation either with ATP or microbiological count when used to monitor cleaning and disinfection of surfaces.⁽²³⁾ However, an investigation conducted in Sweden showed a positive correlation, although marginal, between visual inspection and ATP.⁽²²⁾

Visual inspection remains the most common method to evaluate the effectiveness of cleaning and disinfection of highly touched clinical surfaces. Nevertheless, the findings of the present study prove that visual inspection itself is not enough to guarantee the quality of the process and that it is necessary to record the level of cleaning and disinfection using quantitative methods.⁽²²⁾

Despite its limitations, visual inspection still plays an important role in the esthetic evaluation of the facility and may identify deteriorated areas of surfaces and equipment, which are possibly microorganism reservoirs.⁽¹⁶⁾

Regarding the proportion of surfaces considered dirty when assessed using ATP bioluminescence and ACC, the percentages were similar. There was a significant correlation, although discrete, between the ATP quantification and microbial count methods for the reception desk (rho=0.598; p=0.002) and the stretcher (rho=0.422; p=0.040).

Quantification of ATP using bioluminescence showed a significant result when compared with

the ACC gold standard (p=0.044) and high sensitivity. In this type of analysis, it is suggested that surfaces with an ATP level equal to or lower than 49 RLUs can be considered approved. However, it is important to emphasize that this cutoff is only a guiding reference for the surfaces examined in the present study, and that several other surfaces need to be evaluated for a longer period according to the cleaning protocol applied.

The difficulty to standardize the ATP bioluminescence cutoff hinders comparisons and suggestions. Studies show different results in this regard. An investigation carried out in a basic health unit found a significant correlation for only one surface, the patient stretcher, among five analyzed objects. ⁽²⁴⁾ The authors stressed that ATP quantification is the most suitable method to be used as a parameter when microbial count is considered the gold standard of surface analysis (p<0.001, sensibility of 67%), suggesting 48 RLUs as a cutoff for surfaces to be considered non-approved.⁽²⁴⁾

A similar examination of five surfaces at a Brazilian emergency care unit did not show a correlation between the level of dirt before and after cleaning and disinfection in any surface evaluated using the ATP bioluminescence and ACC methods,⁽¹⁸⁾ with the best cutoff equal to 79 RLUs. This value is lower than the ideal cutoff estimated in a study carried out in Taiwan, equal to 55.7 RLUs.⁽¹⁵⁾

Therefore, based on the analyzed data, the cutoffs of the ROC curve in all the examined studies are lower than 250 RLUs and indicate a tendency toward using lower values, such as 100 RLUs for a 100 cm² surface.

Some aspects may be pointed out to explain the variation in the correlation between ATP bioluminescence and ACC. One of them is that, when a surface has low microbial contamination, the evaluation is more susceptible to errors, given that ATP is the basic source of energy for every vegetal, animal, and microbial cell and, consequently, its presence in environmental surfaces gives an estimate of the quantity of organic matter, including microbial contamination.⁽¹³⁾ A surface can contain organic matter in abundance, but this does not necessarily imply a high microbial density, and vice versa.

Additionally, varying ATP measurements can be explained by the presence of products such as disinfectants, which requires a correct drying process so surfaces can be evaluated later.⁽⁹⁾ Despite this fact, the lack of correlation with specific pathogens cannot be considered a flaw of the ATP bioluminescence method, given that a high RLU value is a warning to improve cleaning and disinfection.⁽¹⁶⁾

The results of the present study allow to infer that it is necessary to combine monitoring methods in outpatient settings, because it provided an opportunity to mitigate the negative points of each type of procedure. Objective feedback about cleaning and disinfection of surfaces is fundamental for the continuing education of healthcare teams regarding the recommended practices of daily cleansing.⁽¹⁰⁾ Regular and systematized monitoring over time can be used to create a data bank that would allow to identify discrepant values. This type of monitoring also helps determine trends in cleaning and disinfection over time, indicating flaws in the process or even the risk of an outbreak.⁽²³⁾

It is worth emphasizing that, at present, most of the contact between healthcare professionals and patients occur in outpatient settings.⁽²⁵⁾ Many infection outbreaks were associated with these settings^(25,26) and, consequently, proper cleaning and disinfection of surfaces and objects close to patients are necessary, because outpatient settings offer the same risk of infection as hospitals.⁽²⁷⁾ Several healthcare settings face unique challenges that demand individualized infection control programs.

Important reflections were formulated in the present study, such as the need to implement more than one monitoring method for surface cleaning and disinfection in health services and the similarity in surface approval rates after cleaning and disinfection when evaluated using the ATP bioluminescence and ACC methods. This result indicates that these methods are effective in monitoring cleaning and disinfection of surfaces in outpatient healthcare services and gives resources for new discussions about the RLU cutoff. These aspects are fundamental for nursing and cleaning teams to do their work with higher qualification.

The main limitations of the present study are the facts that analysis was limited to cleaning and disinfection of surfaces at only one institution and covered a restricted period. In addition, although the surfaces sampled using a swab (in the ATP bioluminescence method) and Rodac[®] plates before and after cleaning and disinfection were adjacent, it is possible that different levels of dirt may have occurred in different close areas of the same surface. Last, the luminometer type, the microbiological method applied, the subjectivity of the evaluators, the cleaning and disinfection protocol, and the disinfectant product used at the facility may differ from those in other studies, compromising the quality of data comparison.

Conclusion

The visual inspection method did not show proportions similar to the cluster of non-approved surfaces when compared with other monitoring methods after cleaning and disinfection. However, when proportions are analyzed in combination, similar proportions of dirty surfaces were found only for ATP bioluminescence and ACC before and after cleaning and disinfection. There was a significant, although discrete, correlation between the ATP quantification and microbial count methods for two surfaces. Receiver operating characteristic analysis indicated that ATP quantification showed a significant result in the comparison with ACC. It is suggested that surfaces with ATP levels equal to or lower than 40 RLUs be considered approved in the studied outpatient setting.

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Collaborations

Furlan MCR, Ferreira AM, Rigotti MA, Guerra OG, Frota OP, Sousa AFL, and Andrade D con-

tributed to the study conception, data analysis and interpretation, writing of the manuscript, critical review of its intellectual content, and final approval of the version to be published.

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