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ORIGINAL RESEARCH

Adenosine Triphosphate Bioluminescence Assay versus Microbiological Swab Culture in the Evaluation of Surface Sanitation in a Pediatric Hospital in Romania

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Purpose: This study aimed to compare two rapid testing methods – Adenosine Triphosphate (ATP) quantification and the total Aerobic Colony Count (ACC) by bioluminescence assay with the standard method – microbiological swab culture. The goal was to determine the advantages and limitations of these rapid alternative tests in assessing the sanitary condition of hospital surfaces.

Methods: A total of 88 samples were collected from various surfaces (stainless steel, metal, plastic, Tarkett, ceramic, glass, textile) in medical and surgical wards of a pediatric hospital. For each tested area, one sample underwent standard microbiological culture (25 cm²), and two adjacent samples (each 100 cm²) were analyzed using the ENSURE® TOUCH luminometer (Hygiena): one for ATP quantification (Ultrasnap) and one for ACC determination (Microsnap Total). Surfaces had been disinfected with commonly used agents—hydrogen peroxide, chlorinated products, or peracetic acid.

Results: The Ultrasnap system identified 25 non-conforming samples; Microsnap Total found 13 non-conforming samples, while microbiological culture revealed one non-conforming sample and an additional 11 with low but detectable bacterial presence. The Ultrasnap method recorded a sensitivity of 100%, a specificity of 72.41% and an accuracy of 72.73%. In comparison, the Microsnap Total system showed higher specificity and accuracy (85.05% and 84.09%, respectively), but failed to identify the positive (non-conforming) test, resulting in a 0% positive predictive value.

Conclusion: The study highlighted several advantages of rapid ATP tests, particularly their ease of use, their ability to provide results in just 10 seconds for Ultrasnap and approximately 7 hours for Microsnap Total, and their capacity to accurately identify well-sanitized surfaces. Additionally, Ultrasnap proved to be a sensitive indicator of residual organic matter, enhancing the capacity to distinguish between different sanitation levels and allowing cleaning and disinfection practices to be adjusted in real time, thereby reducing the risk of cross-transmission.

Keywords: bioluminescence, ATP, disinfection, rapid assessment, hospital surfaces

Introduction

Hospital surfaces are subjected to a continuous and quick recontamination process. Therefore, in the absence of a strict cleaning and disinfection protocol, hospital surfaces may represent important factors in exogenous nosocomial transmission for exogenous nosocomial transmission. Standard techniques for assessing the effectiveness of decontamination procedures involve sampling with a sterile swab, followed by microbiological culture or the fingerprint method. These methods require incubation, colony growth, and microbial species identification, delaying the result release by at least

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24 hours and allowing microbial load transmission in the meantime.[1] On the other hand, the macroscopic inspection conducted through a checklist cannot objectify the disinfection result. The ultraviolet (UV) lamp check certifies that the decontamination has been carried out, but not if it was effective.

In this context, rapid bioluminescence tests measuring adenosine triphosphate (ATP) have been developed. Initially introduced in the 1960s and later implemented in the food industry in the 1990s, they ultimately achieved a breakthrough in medical assistance facilities after 2010.[2–4] Due to their ease of use and rapid results, they are widely employed for assessing cleanliness in food processing areas (cutting boards, slicers, food preparation zones) and hospital high nosocomial risk areas (intensive care units, surgical departments, hematology-oncology, hemodialysis). They are also used for frequently touched surfaces (bed controls, consoles, equipment keyboards, nurse call buttons, door handles, IV stands, light switches), bathrooms (faucets, sinks, dispensers, biofilm-prone areas, including as biological indicators for *Legionella* spp. proliferation), and for evaluating the hygiene of medical staff hands, as well as endoscopic and surgical instruments.[5–15]

ATP is an organic compound, precursor of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), with a role in supplying the energy needed for muscular contraction, nerve impulse propagation, and chemical synthesis. It is found in animals, plants, bacteria, fungi, and both living and dead organic matter. ATP quantification using luminometers relies on the enzymatic reaction between ATP and luciferin, catalyzed by luciferase in the presence of oxygen. This reaction leads to the oxidation of luciferin to oxyluciferin, thereby generating an emission of light directly proportional to the amount of ATP present in the sample. This resulting bioluminescence is measured in relative light units (RLU).[16]

Conventional ATP assays have limitations due to the possibility of ATP degradation to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) – under the action of heat, enzymatic activity, and acidic or alkaline substances. To address this, an advanced A3 test has been developed, capable of detecting ATP, ADP and AMP simultaneously.[17] Additionally, ATP tests cannot directly detect viral contamination on surfaces, as viruses do not synthesize ATP molecules. They are less efficient at detecting gram-positive bacteria compared to gram-negative bacteria, and they cannot accurately predict the presence of microorganisms. Therefore, ATP testing serves as an indirect method for rapid hygiene assessment.[5]

The ENSURE ® TOUCH (Hygiena) system, launched in 2019, works as a luminometer, but also includes the Microsnap Total component, which allows the determination of the Aerobic Colony Count (ACC) and even the detection of *Enterobacterales*, coliform bacteria, *E. coli, Salmonella enterica, Legionella* spp. (using corresponding consumables).[18]

In Romania, the prevalence of healthcare-associated infections (HAIs) was 3.1%, according to the 2023 Point Prevalence Survey on HAIs and antimicrobial use in acute care hospitals. In comparison, the European average was 7.1% [95% CI 7.0–7.2%], suggesting a substantial likelihood of underreporting in Romania.[19,20] Since 2021, according to the Order of the Ministry of Health 1761/03.09.2021, rapid ATP-based techniques have been introduced as standard methods for surface sanitation assessment in nosocomial environments. Bacteriological testing remains reserved for epidemiological investigations, for establishing the source and transmission route during outbreaks, as well as for evaluating work protocols and infection control measures.[21,22] However, most hospitals continue to evaluate the effectiveness of cleaning/disinfection activities using a checklist and standard microbiological swab culture.

The aim of the study was to compare two rapid methods—adenosine triphosphate quantification and total aerobic colony count using the ENSURE® TOUCH luminometer (Hygiena)—with the standard microbiological swab culture, in order to evaluate their advantages and limitations in assessing the hygienic status of hospital surfaces. The study also aimed to highlight differences based on surface type, class of used disinfectants, the time interval between decontamination and sampling, and the associated cost of each method.

Materials and Methods

Study Design

A unicentric, cross-sectional study was conducted to monitor surface sanitation levels from different wards of "Louis Țurcanu" Children's Emergency Clinical Hospital Timișoara, between March 1 – May 1, 2024. "Louis Țurcanu" Children's Emergency Clinical Hospital Timișoara is the leading tertiary medical unit with a pediatric profile in the West of Romania, with a total number of 501 beds (73 surgical ones), with specialties covering all child and adolescent

pathologies. These include pediatric medical specialties, such as neonatology, nutritional rehabilitation, oncohematology, hemodialysis, and palliative care, as well as surgical specialties, which encompass three operating rooms and services in general surgery, plastic surgery, orthopedics, otolaryngology, ophthalmology, and intensive care. The hospital also features an Emergency Department and a Bone Marrow Transplant Unit.

Sample Collection

Samples were collected from any surface considered to be clean, without visible traces (swabbing a visibly dirty surface inhibits the bioluminescence reaction and may generate incorrect results). The collection was performed by the same person from the HAIs Prevention and Control Department staff. All samples were collected after a minimum of 30 minutes since disinfection to allow the completion of the biocidal effect (including the sporicidal and tuberculocidal ones) and to allow the surface to dry, thereby minimizing the interference between residual chemical waste and the bioluminescence reaction.

From each sampling site, three types of samples were collected simultaneously from adjacent areas:

• 1 sample for microbiological culture to assess the bacterial load,

• 2 samples for rapid sanitation testing using the ENSURE ® TOUCH (Hygiena) luminometer:

- one for the quantification of adenosine triphosphate, targeting both live and dead organic matter left on surfaces, using Ultrasnap swabs;

- one for the detection and quantification of the total Aerobic Colony Count, using Microsnap Total swabs.

The microbiological sample was obtained using a sterile cotton swab, dampened in 1 mL of sterile saline solution. The swab was used to wipe a 25 cm^2 area defined by a sterile template (5x5 cm – for flat surfaces), primarily targeting high-contact surfaces or areas likely to be exposed to biological fluids. These included regular and irregular surfaces, regardless of the material – stainless steel, metal, plastic, Tarkett, ceramic, particle board, glass, textile, and others (oilcloth, oil-painted walls).

Samples analyzed with the ENSURE ® TOUCH (Hygiena) luminometer were collected from a 100 cm^2 surface (10x10 cm; in case of irregular shapes, a similar surface was swabbed to enable result comparability) using the Ultrasnap test and read within 30 seconds after activation.[23] An additional 100 cm^2 sample, adjacent to the microbiological sampling area, was collected using an MS1-Total swab (enrichment consumable) to determine the total Aerobic Colony Count (ACC).

It is worth mentioning that the collection of the three samples from the exact same area could not be achieved, because the producer mentions not to swab the same surface multiple times in order not to lower the biological load after the first wipe.[24]

The calibration of the ENSURE® TOUCH (Hygiena) luminometer was verified using the Positive Control Kit, along with a negative control reading (blank sample) performed prior to sample collection.

Sample Analysis

A 1/10 dilution and a 1/100 dilution were prepared from the homogenized physiological serum in which the swab intended for standard microbiological culture had been discharged. From each dilution, 0.1 mL were dispersed on a separate plate of Columbia Agar + 5% blood (BioMaxima) and MacConkey medium (BioMaxima) and incubated at 37°C. The following formula was applied: $CFU/cm^2 = N1 \times D1 \times \text{dispersed quantity}/25\ cm^2 + N2 \times D2 \times \text{dispersed quantity}/25\ cm^2$ (where N1, N2 represent the colony forming units on each plate; D1, D2 represent dilutions used for each Petri plate).[25] For samples that presented bacterial growth, species identification was performed using the Vitek 2 Compact (BioMerieux) automated system within the hospital laboratory.

The Microsnap1-Total swab, after releasing the specific enrichment broth from the Snap-Valve, was incubated for 7 h at 37°C in a Hygiena Mini Incubator. Following that, 0.1 mL of the sample was transferred to the detection device (MS2-TOTAL), activated, homogenized, and read using the ENSURE® TOUCH device (Hygiena).[18]

A conforming sample (signifying adequate surface hygiene) was defined:

1. By microbiological method – a surface from which less than five colony forming units (CFU)/cm^2 were isolated in the absence of potentially pathogenic germs;[25]

2. By Ultrasnap – a result of less than 20 RLU. Values between 21–59 RLU were considered to be borderline, and over 60 RLU were considered non-conforming due to biological contamination, requiring repeated disinfection;[23]

2. By Microsnap Total – <500 RLU, which, by conversion, means <500 CFU, meaning <5 CFU/ cm^2.[26]

Data Analysis

For the comparison of the results obtained from rapid ATP-based tests with those from standard microbiological culture method, the following metrics were assessed: true positive (non-conforming), true negative (conforming), false positive and false negative.

Sensitivity was calculated as the ratio between true positive results (non-conforming results which coincide in standard culture and rapid tests – Ultrasnap/Microsnap Total) and positive results identified by microbiological culture, while the specificity was calculated as the ratio between the true negative results (conforming results which coincide with standard culture and rapid ATP tests) and the total negative results obtained by culture.[27]

The positive predictive value (PPV) (the probability of a non-conforming sample, identified by rapid tests, to be confirmed by standard method – microbiological culture) was calculated as the ratio between non-conforming results concordant between both methods to the total number of non-conforming results identified by the rapid test. The negative predictive value (NPV) (the probability of a conforming sample, identified by rapid tests, to be confirmed by standard method – microbiological culture) was calculated as the ratio between conforming results concordant between both methods to the total number of conforming results identified by the rapid test. Test accuracy, meaning the overall performance of rapid ATP tests, taking into consideration the number of results confirmed by microbiological culture, was calculated as the sum of true positive and negative results divided by total number of samples analyzed (the sum of true and false positive and negative results).[28]

The cost per sample was calculated based only on the value of the consumables used for each test.

The study was reviewed by the Ethics Committee for Scientific Research of "Louis Țurcanu" Children's Emergency Clinical Hospital Timișoara and received Approval No. 21034/December 21, 2023.

Statistical Analysis

The database was analyzed using the IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL) software program. Numeric variables were expressed by median and interquartile range (IQR), while nominal variables by value and percentage. Data distribution testing was carried out using the Shapiro–Wilk test. Nominal variables were compared using the chi-square test (Fisher's exact test). For correlation, the Spearman rho Correlation coefficient was applied. The statistical significance threshold value was considered to be ≤ 0.05, and only two-tailed tests were used.

Results

In this study, between March and May 2024, 90 samples were collected for the microbiological control of surfaces, together with 90 Ultrasnap tests and 90 Microsnap Total tests. There were 2 excluded samples, which failed the Ultrasnap test readings (probably due to delayed reading after sample activation), along with their corresponding paired tests. Thus, 88 surface samples were included in the final analysis and investigated by bioluminescence quantification methods (Ultrasnap and Microsnap Total system) and by microbiological culture. Distribution of nosocomial surface tests is shown in Table 1:

The percentage of non-conforming samples identified by Microsnap Total was statistically significant and higher in the medical wards compared to surgical wards ($p = 0.016$). This difference is less obvious in case of Ultrasnap samples ($p = 0.062$) and was not observed in standard microbiological culture ($p = 1$). The laboratory identified only one non-conforming sample, collected from a Pediatrics Diabetology patient room, from a (plastic) bed frame, by identifying a low bacterial load (0.3 CFU/cm²) but with the presence of *Escherichia coli*. For this sample, the Microsnap Total result was within conforming limits (<20 CFU), whereas the Ultrasnap test indicated a slight excess of organic material (63 RLU), classifying it as non-conforming.

The number of non-conforming samples was statistically significant and higher in the Pediatric ward, compared to Hemodialysis or Orthopedics ($p = 0.013$) among Ultrasnap samples. Microsnap Total tests also indicated a significantly higher percentage of non-conforming samples in Pediatrics, compared to Oncohematology ($p = 0.006$).

The time interval between surface disinfection and sample collection had a median of 223 minutes [IQR: 856 minutes], with a minimum of 30 minutes and a maximum of 1786 minutes.

Table 1 Test Repartition Based on the Profile of the Ward Where They Were Collected From

Ward	No. of Collected Samples/ Test Type	Ultrasnap		Microsnap Total		Microbiological Culture	
		No. of Conforming and Borderline Samples <60 RLU	No. of Non-Conforming Samples ≥60 RLU	No. of Conforming and Borderline Samples <500 RLU	No. of Non-Conforming Samples ≥500 RLU	No. of Conforming Samples <5CFU/cm^2	No. of Non-Conforming Samples ≥5CFU/cm^2
Medical profile (pediatric) [n,(%)]	64(100)	42(65.63)	22(34.38)	51(79.68)	13(20.31)	63(98.43)	1(1.56)
Surgical/ICU profile (pediatric) [n,(%)]	24(100)	21(87.50)	3(12.50)	24(100)	0(0)	24(100)	0(0)
Total	**88(100)**	**63(71.59)**	**25(28.41)**	**75(85.23)**	**13(14.77)**	**87(98.86)**	**1(1.13)**
Pediatrics [n,(%)]	24(100)	12(50.00)	12(50.00)	15(62.50)	9(37.5)	23(95.83)	1(4.17)
Oncohematology [n,(%)]	16(100)	11(68.75)	5(31.25)	16(100)	0(0)	16(100)	0(0)
Pediatric Pneumology [n,(%)]	8(100)	5(62.50)	3(37.50)	6(75.00)	2(25.00)	8(100)	0(0)
Neonatology [n,(%)]	8(100)	6(75.00)	2(25.00)	6(75.00)	2(25.00)	8(100)	0(0)
Hemodialysis [n,(%)]	8(100)	8(100)	0(0)	8(100)	0(0)	8(100)	0(0)
ICU [n,(%)]	8(100)	6(75.00)	2(25.00)	8(100)	0(0)	8(100)	0(0)
Surgery [n,(%)]	8(100)	7(87.50)	1(12.50)	8(100)	0(0)	8(100)	0(0)
Orthopedics [n,(%)]	8(100)	8(100)	0(0)	8(100)	0(0)	8(100)	0(0)

Abbreviations: ICU, Intensive care unit; RLU, relative light units; CFU, colony forming units.

The correlation between decontamination time to sampling was indirect, weak but statistically significant for Microsnap Total tests (rho = −0.276, p = 0.009), and with no statistical significance for Ultrasnap tests (rho = −0.201, p = 0.060).

The median value of Ultrasnap results was 26.50 RLU [IQR: 73], with a minimum value of 0 and a maximum value of 1864 RLU, and for the Microsnap Total tests, the average was <20 CFU [IQR: 112], with a minimum of <20 and a maximum of 70,677 CFU.

The distribution of sample according to surface type, construction material, type of biocide used for decontamination, and the time interval between disinfection and sample collection is presented in Table 2.

Statistically significant differences were noted in the following situations:

- number of samples with organic load over the permitted threshold, identified by Ultrasnap, was higher among the ones collected off of metal than the ones collected off of plastic (p = 0.025), laminated particle board (p = 0.035) or glass (p = 0.001);

- number of non-conforming samples, identified by Ultrasnap, was higher among the ones collected off of stainless steel, compared to the ones collected off of glass (p = 0.028);

- likewise, the percentage of non-conforming samples, collected with Ultrasnap swabs off of textiles, was higher compared to the one of samples harvested off of glass (p = 0.005);

- percentage of non-conforming samples, collected by Ultrasnap test within the first hour after decontamination was statistically significant higher than that of the samples collected in the timespan of 4–24 h after decontamination (all of them after nebulization – p = 0.026).

The Microsnap Total swab tests revealed a higher number of samples with ACC above the conformity threshold in hallways versus patient rooms (p = 0.018) or bathrooms (p = 0.008). Surfaces disinfected with chlorine-based biocides showed a statistically significantly higher microbial load than those treated with peracetic acid-based disinfectants (p = 0.043). Moreover, the number of non-conforming samples was significantly higher following routine (current) decontamination compared to terminal decontamination (p = 0.016).

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Table 2 Analysis of Conforming/Non-Conforming Samples by Bioluminescence-Based Methods According to Collection Variables

Surface	No. of Collected Samples/Test Type	Ultrasnap		Microsnap Total		Microbiological Culture	
		No. of Conforming and Borderline Samples <60 RLU	No. of Non-Conforming Samples ≥60 RLU	No. of Conforming and Borderline Samples <500 RLU	No. of Non-Conforming Samples ≥500 RLU	No. of Conforming Samples <5CFU/cm^2	No. of Non-Conforming Samples ≥5CFU/cm^2
Surface Type							
Plastic [n,(%)]	25(28.41)	19(76.00)	6(24.00)	21(84.00)	4(16.00)	24(96.00)	1(4.00)
Laminated particle board [n,(%)]	13(14.77)	10(76.92)	3(23.08)	10(76.92)	3(23.08)	13(100)	0(0)
Stainless steel [n,(%)]	12(13.64)	8(66.67)	4(33.33)	11(91.67)	1(8.33)	12(100)	0(0)
Glass [n,(%)]	15(17.05)	15(100)	0(0)	15(100)	0(0)	15(100)	0(0)
Textile [n,(%)]	7(7.95)	4(57.14)	3(42.86)	5(71.43)	2(28.57)	7(100)	0(0)
Tarkett [n,(%)]	5(5.68)	3(60.00)	2(40.00)	4(80.00)	1(20.00)	5(100)	0(0)
Ceramic [n,(%)]	5(5.68)	3(60.00)	2(40.00)	4(80.00)	1(20.00)	5(100)	0(0)
Metal [n,(%)]	3(3.41)	0(0)	3(100)	3(100)	0(0)	3(100)	0(0)
Others [n,(%)]	3(3.41)	1(33.33)	2(66.67)	2(66.67)	1(33.33)	3(100)	0(0)
Sampling area							
Devices [n,(%)]	9(10.23)	7(77.50)	2(22.22)	9(100)	0(0)	9(100)	0(0)
Bathroom Surfaces [n,(%)]	11(12.5)	9(81.82)	2(18.18)	11(100)	0(0)	11(100)	0(0)
Bedding [n,(%)]	7(7.95)	4(57.14)	3(42.86)	5(71.43)	2(28.57)	7(100)	0(0)
Hallway Surfaces [n,(%)]	4(4.54)	1(25.00)	3(75.00)	1(25.00)	3(75.00)	4(100)	0(0)
Food contact surfaces [n,(%)]	2(2.27)	1(50.00)	1(50.00)	2(100)	0(0)	2(100)	0(0)
Patient Room Surfaces [n,(%)]	55(62.5)	41(74.55)	14(25.45)	47(85.45)	8(14.54)	54(98.18)	1(1.82)
Product with which disinfection was conducted							
Disinfectants based on hydrogen peroxide [n,(%)]	53(60.23)	37(69.81)	16(30.18)	45(84.90)	8(15.09)	52(98.11)	1(1.88)
Disinfectants based on peracetic acid [n,(%)]	16(18.18)	14(87.50)	2(12.50)	16(100)	0(0)	16(100)	0(0)

Chlorine Disinfectants [n,(%)]	16(18.18)	9(56.25)	7(43.75)	11(68.75)	5(31.25)	16(100)	0(0)
Alcohol-based Window Disinfectants [n,(%)]	3(3.41)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0)
After terminal decontamination [n,(%)]	24(27.27)	21(87.50)	3(12.50)	24(100)	0(0)	24(100)	0(0)
After current decontamination [n,(%)]	64(72.73)	42(65.62)	22(34.37)	51(79.68)	13(20.31)	63(98.44)	1(1.56)
Time interval between decontamination and collection							
30–60 minutes [n,(%)]	8(9.09)	3(37.50)	5(62.50)	8(100)	0(0)	8(100)	0(0)
60–119 minutes [n,(%)]	16(18.18)	10(62.50)	6(37.50)	11(68.75)	5(31.25)	15(93.75)	1(6.25)
120–239 minutes [n,(%)]	37(42.04)	28(75.67)	9(24.32)	31(83.78)	6(16.22)	37(100)	0(0)
4h-24 h [n,(%)]	19(21.59)	16(84.21)	3(15.78)	17(89.47)	2(10.52)	19(100)	0(0)
>24 h [n,(%)]	8(9.09)	6(75.00)	2(25.00)	8(100)	0(0)	8(100)	0(0)

Notes: Others - oilcloth, oil painted walls.
Abbreviations: RLU, relative light units; CFU, colony forming units.

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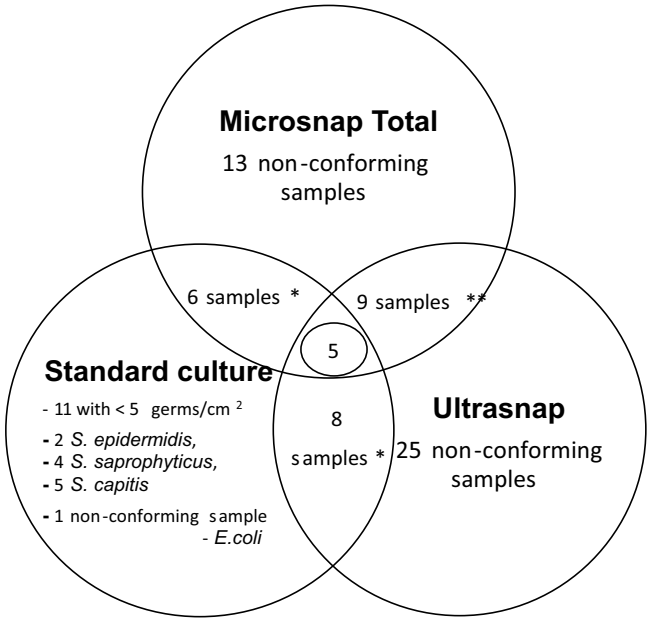

Figure 1 Concordance of samples with bacterial load in standard culture and non-conforming ones from Ultrasnap/Microsnap Total rapid.
Notes: * non-conforming samples on Ultrasnap/Microsnap, which have shown bacterial growth on standard culture; ** non-conforming samples on Ultrasnap and Microsnap Total. Microbiological culture: non-conforming samples ≥5CFU/cm$^{2.}$ Ultrasnap: non-conforming samples ≥60 RLU. Microsnap Total: non-conforming samples ≥500 RLU.

Out of the 12 samples that exhibited bacterial growth identified by microbiological culture (<5 CFU/cm^2), 6 samples were classified as non-conforming by Microsnap Total test, 8 samples were non-conforming according to the Ultrasnap test, and 5 samples were non-conforming for both rapid ATP tests, as is highlighted in Figure 1.

The comparison between the standard microbiological culture method and the rapid tests revealed very high negative predictive values for both rapid tests, but with very low positive predictive values. Ultrasnap testing accuracy was lower compared to the quantification of the total number of viable aerobic bacteria using Microsnap Total (Table 3).

The consumable cost per sample was € 3.6 for the quantification of ATP using the Ultrasnap luminometer test, € 7 for aerobic colony count determination using the Microsnap Total test and € 0.7 without, and € 7.29/sample with bacterial growth (cost excluding antibiogram) for the microbiological control of surfaces.

Table 3 Analysis of Rapid ATP Tests in Comparison to the Standard Method – Microbiological Culture

	Ultrasnap	**Microsnap Total**
Sensitivity (95% CI)	1/1 100%	0/1 0%
Specificity (95% CI)	63/87 72.41% (61.79–81.46)	74/87 85.05 (75.80–91.80)
Positive Predictive Value - PPV (95% CI)	1/25 4% (0.10–20.00)	0/13 0%
Negative Predictive Value – NPV (95% CI)	63/63 100%	74/75 98.67 (92.79–99.97)
Accuracy (95% CI)	64/88 72.73 (62.19–81.68)	74/88 84.09 (74.75–91.02)

Abbreviation: CI, confidence interval.

Discussion

In a hospital, the cleaning and disinfecting activity represents the foundation of healthcare-associated infection prevention and control. Even more so in a pediatric hospital, where certain wards care for highly vulnerable patients, including those with an immature immune system (neonatology, premature babies), immunosuppressed patients (in Oncohematology, HIV compartment, dystrophic patients), and patients undergoing highly invasive therapeutic interventions (in surgical wards, intensive care unit, hemodialysis). These factors collectively increase the risk of pathogen transmission. Physiological and behavioral characteristics of children also play a part in facilitating the spread of infections – sphincter control immaturity, probing the environment by mouth or hand touch, excessive salivation, pacifier use, direct and frequent contact with other possible infection sources (other children, caregivers, healthcare staff), playing with toys and inappropriate hand hygiene due to young age or lack of education.[29–32] Family members, indispensable for the emotional comfort children, may also act as vectors, introducing community-acquired infections into the hospital or contributing to the spread of nosocomial infections within the general population. Furthermore, the use of diapers, without adherence to personal and collective hygiene rules, poses significant risks of hand contamination for healthcare staff, relatives, but also surfaces in the nosocomial environment.

Under such conditions, the surface recontamination rate is higher, forcing intensified cleaning and disinfection efforts, as well as enhanced monitoring of their effectiveness.[33] The hospital involved in this study routinely conducts microbiological monitoring of surface hygiene; however, its use of rapid testing methods is limited, with a preference for qualitative assessments over ATP quantification. To avoid bias and general reactivity, neither the cleaning nor the laboratory staff was informed about the use of duplicate monitoring methods during this study. In these circumstances, 25 samples (28.41%) were classified as non-conforming by Ultrasnap, 13 samples (14.77%) by Microsnap Total, and only 1 sample (1.13%) by standard microbiological culture.

Among the 11 other samples that exhibited low bacterial loads (between 0.3 and 1.5 CFU/cm² of *Staphylococcus spp.* - including *S. epidermidis, S. saprophyticus*, and *S. capitis*), all were considered conforming. These samples were distributed across several wards: Pediatrics (4), Oncohematology (4), Pneumology (2), and Neonatology (1). The surfaces sampled included injectomats (3), bed linens (2), ward and bathroom surfaces (4 - including ceramic, laminated wood, metal, and stainless steel), and plastic hallway surfaces (2). All these surfaces were currently disinfected with chlorine-based products (4), peracetic acid (4), hydrogen peroxide (3). Most surfaces had been disinfected 30–60 minutes (4 samples) or 120–239 minutes (6 samples) prior to sampling.

The lack of correlation between RLU values obtained from simple ATP measurements and CFU values identified by standard culture is not unexpected. One of the explanations is the persistence of ATP after cellular death, with the possibility of its quantification in lysed cells. Likewise, luminometers can also determine some organic residues (blood, secretions, food) that can be present on certain surfaces, which determines the rise of the RLU value, divergently to CFU (in cases when disinfection was effective, but the cleaning was poor).[34] Another possible explanation is the presence of viable but non-culturable germs–microorganisms that cannot grow on standard culture media or that require specific growth conditions. These organisms may go undetected by culture but still contribute to increased RLU values. This explanation can be used as well for the significant discrepancies observed between the microbiological results and those obtained by the Microsnap Total system. Standard culture results revealed no significant differences in surface contamination levels between medical and surgical wards. In contrast, rapid ATP tests have revealed a superior level of surface sanitation in surgical wards, clearly visible with the Microsnap Total tests ($p = 0.016$).

Regarding the timing of sample collection, 60.22% of them were collected in a 1–4 hour interval after disinfection, aligning with the typical schedule of the hospital's HAI Prevention and Control team, who operate between 10:00 and 11:00 a.m. to avoid disrupting morning ward round. All samples collected after 240 minutes came from spaces, which underwent terminal decontamination, which remained closed between nebulization and sampling.

This context may explain the weak but statistically significant inverse correlation between disinfection-to-sampling time and microbial load, particularly for Microsnap Total results. This aspect, in conjunction with the fact that the percentage of non-conforming samples, identified by Microsnap Total (collected after terminal decontamination), is statistically significant

and smaller than the percentage of non-conforming samples collected after the current decontamination, supports the hypothesis of suboptimal efficacy of routine cleaning procedures, especially within the Pediatric ward.

Literature presents the existence of possible interferences with chemical substances (lactic acid, trisodium phosphate, sodium hydrochloride) or disinfectants (hydrogen peroxide, sodium hypochlorite) which can inhibit the bioluminescence chemical reaction, generating biases.[1,35] In this context, a question was raised regarding whether hydrogen peroxide, used in terminal decontamination, had not interfered and generated false negative results in ATP measurement. This hypothesis was dropped as all Microsnap Total results had a value <20 CFU, and the cultures did not show bacterial growth.[36] Despite the lower probability of residual microbial contamination following terminal cleaning, verification of such spaces remains essential, particularly because they are categorized as high-risk nosocomial environments. In this study, 3 samples (12.50%) of the surfaces presented biological load over the admitted threshold (Ultrasnap), after the completion of terminal decontamination – the keyboard of a vital signs monitor, a treatment table (in the same intensive care room) and a bed rail from a surgical patient room.

The median of the Microsnap Total fell below the compliance threshold value. However, for the Ultrasnap tests, the median fell in the range of borderline sanitation values, suggesting the need to intensify cleaning efforts and to consider collecting ATP samples sooner after disinfection, to better capture post-cleaning effectiveness before recontamination occurs. In terms of material, the study clearly shows that flat, non-porous surfaces such as glass are associated with lower organic loads, whereas surfaces made of stainless steel, metal, textiles, or other porous materials showed elevated RLU values. This is likely due to the tendency of these surfaces to become scratched, absorbent, or more difficult to clean effectively.[37]

The Microsnap Total system has allowed highlighted higher percentages of non-conforming samples in high-traffic areas such as hallways, where frequent recontamination may occur and where sanitation efforts may be less consistent compared to patient rooms, bathrooms, or medical equipment. While ATP bioluminescence assays cannot evaluate disinfectant efficacy directly, Microsnap Total – by determining viable aerobic colony counts – revealed a significantly higher number of non-conforming samples following the use of chlorine-based disinfectants, compared to those treated with peracetic acid-based products.

The study revealed excellent negative predictive values for both tests (Ultrasnap 100%/Microsnap Total 98.67%), indicating a high probability that surfaces classified as conforming by rapid tests were also confirmed by standard microbiological culture. Specificity was acceptable, especially for Microsnap Total (72.41%/85.05). However, the positive predictive values were low (4%/0%), suggesting that rapid ATP testing is more reliable for confirming clean surfaces than for detecting contamination, a similar conclusion to one of the 2023 study conducted in a pediatric clinic by the team led by Lucas de Oliveira Bernardes.[38] Regarding surfaces with organic load, ATP quantification using a luminometer was more adequate. In contrast, Microsnap Total showed 0% sensitivity, indicating a high risk of false negatives results – failing to detect low levels of viable but pathogenic bacteria, such as Gram-positive cocci, which may still be isolated in standard culture. Prior research estimates that only 33% of ATP detected in a hospital environment is attributable to bacterial presence, with the remainder originating from non-microbial organic matter.[39]

On the other hand, the great number of non-conforming samples identified by the Microsnap Total system versus standard culture can be explained through the fact that liquid enrichment media facilitate bacterial access to nutritive substances more and, consequently, the growth, as opposed to solid media, where bacteria access to nutrients is more limited.[40]

Study Limitations

Sampling was performed on adjacent, not identical, surface areas, introducing potential bias, as repeated swabbing of the same area is discouraged due to the significant reduction in biological load after initial contact. The big interval between disinfection and collection, allowed for surface recontamination and elevated RLU value, but the choice of maintaining the hospital collection schedule was made in order to see the utility of rapid ATP tests in the context of current organizational conditions. Strictly over the course of the study, no microbiological samples were submitted for external evaluation. However, the hospital adheres to the regulatory standards and quarterly checks at the Timiș Public Health Department the results of 10 surface samples. No significant discrepancies between the hospital laboratory and the public health authority have been reported to date.[41] The short duration of the cross-sectional study and the small group of samples per the testing method, may have influenced the sensitivity/specificity calculation for rapid ATP tests.

Conclusion

The study highlighted several advantages of rapid ATP tests, particularly their ease of use, their ability to provide results in just 10 seconds for Ultrasnap and approximately 7 hours for Microsnap Total, and their capacity to accurately identify well-sanitized surfaces. Additionally, Ultrasnap proved to be a sensitive indicator of residual organic matter, enhancing the capacity to distinguish between different sanitation levels and allowing cleaning and disinfection practices to be adjusted in real time, thereby reducing the risk of cross-transmission.[42]

Rapid ATP tests are easy to introduce into routine surveillance of the nosocomial environment and increase the accountability of the cleaning and auxiliary staff, which represent an important link in the healthcare-associated infections prevention and control program.

Total Microsnap combines some of the advantages of standard microbiological culture with the benefit of same-day results and demonstrated superior specificity/precision compared to Ultrasnap, which does not differentiate live germs from organic wastes. While microbiological culture is an easy method to implement in a hospital with a microbiology laboratory, it exhibited the lowest discriminatory ability between surfaces with residual organic load and clean ones.

The comparable cost between ACC determination using bacterial culture and the rapid method (particularly in positive samples) further supports the use of culture – when we need a genus-level or species-level identification, and the use of Microsnap Total – when the main goal is to assess the cleanliness of surfaces in healthcare settings.

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Author Contributions

All authors made significant contributions to the reported work, whether in conception, study design, execution, data acquisition, analysis, and interpretation, or across all these areas. They participated in drafting, revising, or critically reviewing the article. All authors approved the final version for publication, agreed on the submission journal, and accepted accountability for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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