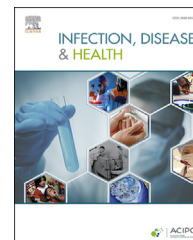


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Research paper

Using a simplified ATP algorithm to improve data reliability and improve cleanliness standards for surface and medical device hygiene

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Abstract *Background:* An algorithm has been improved to mitigate variability in cleanliness measurements of various surfaces using rapid Adenosine Triphosphate (ATP) testing. A cleaning intervention step (CIS) verifies the cleanability of those surfaces.

Methods: ATP testing was performed on surfaces which were pre-approved as “clean” and ready for re-use. Adjacent (duplicate) ATP sampling was undertaken on 421 environmental surfaces, medical devices and other implements. The CIS was conducted on 270 surfaces using an aseptic technique and disposable cleaning wipes.

Results: The two initial ATP results were plotted against each other with a 100 RLU threshold grading the results as clean ($2x < 100RLU$), dirty ($2x > 100RLU$) or equivocal ($1x < 100RLU$ and $1x > 100RLU$). Of the surfaces sampled, 68.5 % were clean (288/421), 13.5 % were dirty (57/421) and 18 % were equivocal (76/421). The duplicate testing demonstrated a false negative rate of 10 % (44/421) where the first swab was <100 RLU and the second swab >100 RLU. For the equivocal group, the gap between the two swabs was >100 RLU for 7.5 % of surfaces (33/421). The CIS was conducted on 270 of the surfaces tested and showed that cleaning could be improved ($P = <0.001$) on 88.5 % of surfaces (239/270).

Conclusion: The simplified ATP testing algorithm provides real-time discrimination between surface cleanliness levels and improved certainty over surface hygiene. The duplicate swab sampling approach mitigates uncontrolled variability in the results and the CIS provides a nuanced understanding of the measurable cleanliness of any surface.

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Highlights

- A simplified ATP algorithm is proposed and tested to improve data reliability with rapid ATP testing.
- A duplicate sampling method combined with a Cleaning Intervention Step (CIS) validates the initial data in real time.
- The simplified ATP algorithm provides a three-tiered classification system for surface cleanliness measurements.
- The simplified ATP algorithm with the CIS allows a more nuanced approach to surface hygiene based on credible data.

Introduction

The seminal paper on rapid testing for adenosine triphosphate (ATP) identified that rapid ATP testing could transform quality assurance of cleaning processes within healthcare settings [1]. There are now two decades of additional studies with many positive findings and a growing acceptance of this rapid and real time cleanliness monitoring tool [2]. But despite the growing evidence, there have been barriers which have prevented the uptake of this simple to use tool [3]. A newly proposed method of ATP sampling, formed into a process algorithm, is a practical way to unscramble often conflicting cleanliness data obtained through ATP testing, and this study improves upon the earlier version by simplifying the procedure [4].

Despite the initial hope for widespread adoption of ATP testing, several early reviews outlined an array of difficulties [5]. Validation papers have fallen short of a practical solution to using rapid ATP testing [6–8].

The difficulties with rapid ATP testing can be loosely divided into four categories as follows.

Firstly, whilst the relative light units (RLU) measurement scale is used by the various device manufactures, the scaling is truly relative, and every brand of ATP testing device uses its own unique scaling [9]. Although there is no single scale to a quantitative measurement of ATP, brand testing comparisons demonstrate similar levels of linearity and lower limits of detection (LLD) [6,9,10].

This idiosyncratic (branded) relative scaling prevents any interoperability between device brands and diminishes comparability between studies where differently branded ATP devices are used. This lack of common measurement scale is a barrier to acceptance and leads to misunderstanding with users who have acquired different brands from those used in reference papers.

Secondly, there is a significant lack of precision when looking for closely comparable samples containing ATP, whether in a pure form or in suspension cultures [11]. This inherent variability is quite high (the coefficient of variance [Cv] is often as high as $Cv = 0.4$). This variability across similar field samples confounds the interpretation of results. Whilst variability is a concern, the lower limit of detection for most rapid ATP testing devices is quite low, and therefore the results are quite sensitive. However, the lowest limit of quantitation (LLQ) is higher in relative terms for some of the device brands [9,10]. This separation

between LLD and LLQ undermines the interpretation of detectable but very low levels of ATP.

Thirdly, this imprecision is worst at the lower end of the reading scale where cleanliness measurements are most important. At the LLD/LLQ there is also the potential impact of cleaning chemical residues, which can quench the response of the ATP tests [10,12]. The lysis systems contained in some of the brands of consumables also exhibit mixed performance and some bacterial or fungal species may be undetected in normal use [10,11]. The presence of bacterial or other biofilms can also diminish detection [13].

Inability to detect ATP provides a misleading perception of cleanliness which can interfere the potential for correlation with unexpectedly high levels of ATP in field samples. Interpretation of inconsistently high results is problematic and confounding to statistical analysis, particularly where microbial estimation is used in tandem with ATP testing [14,15]. Work has shown that whereas simple dried colonies of bacterial contaminants may be easily wiped away, dry surface biofilms are very difficult to remove through a standard wiping technique [16].

Fourthly, there is always the risk of sampling error. Whilst it might be hoped that cleanliness on a surface would be uniform, this cannot be assumed, and so sampling a particular location immediately adjacent to a deposit of soiling or colony of microbes remains a real concern within healthcare settings [17]. What is needed is a defensible and accessible method to understand unusually and unexpectedly high ATP testing results. Using a sampling approach based on a single swab (per surface) diminishes the ability to understand unexpectedly high results.

The ATP algorithm uses a duplicate initial sampling approach to overcome the problems of a single swab approach, however the subsequent calculations were complicated. This paper outlines a simplified ATP algorithm which still uses initial duplicate sampling, which is used to provide a new three tiered approach to cleanliness classification, i.e. clean, dirty or equivocal. The data demonstrates the importance of a simplified algorithm that preserves a nuanced classification approach to cleanliness measurements and improves data reliability from use of this simplified ATP algorithm.

Methods

ATP testing was conducted within healthcare settings and food preparation areas, sampling environmental surfaces,

medical devices and food preparation implements, all of which were hard surfaces.

A total of 421 discrete test surfaces are included in the results. ATP testing was conducted using a Hygiena rapid ATP testing device (Key Diagnostics, Sylvania, Australia) and associated consumables. The Hygiena ATP testing device was selected because the LLD and LLQ were both matched at the “zero” level reading on the RLU scale of that device allowing a more reliable interpretation at readings at the lowest end of detectability [9].

Duplicate samples were taken on each surface on devices, surfaces and implements that had been already subjected to cleaning and reprocessing for reuse. The swabs were taken immediately sequentially. As the subjected surfaces were all presented as “clean”, the sampling assumed that the cleanliness had been achieved uniformly across the face of the tested surfaces.

A cleaning intervention step (CIS) was undertaken using a disposable single use detergent wipe containing a formulated an anionic/zwitterionic detergent solution (Speedyclean Wipes, Whiteley Corporation, Australia). The wipes were validated not interfere with the ATP consumables. The wipe method was aseptic and each single wipe was used in only one direction, on one surface [19].

The aseptic CIS was conducted over the area of the surface that had been tested with the initial duplicate swabs and was conducted immediately following recording of the initial ATP testing. The post-intervention ATP test was then tested on the cleaned surface once the surface had dried (normally less than 1 min) and the result recorded. The CIS was continued until a final result of <25RLU was achieved. This level of 25 RLU (Hygiena brand only) has been shown as the level at which no difference can be determined between the initial swabs and the CIS [20]. The final CIS result (<25RLU) was used for comparison with the averaged results from the initial duplicate swabs from the same surface.

Data analysis

We present the agreement between the two swabs as a scatterplot. The results of the first swab were plotted along the X-axis and the second swab along the Y-axis. The cleanliness cut-off level was set at 100 RLU on each axis dividing the graph into quadrants [21]. Surfaces fall into the

first quadrant when both swabs are less than 100 RLU (both clean). Surfaces in the fourth quadrant had two swabs of greater than 100 RLU (both dirty). The second and third quadrants show those surfaces where one of the swabs was greater than 100 RLU and the other swab less than 100 RLU.

For statistical analysis much of the data is not normally distributed [Wilks–Shapiro], and so where standard approaches are inappropriate, the Wilcoxon Rank Sum Test has been used.

Statistical analyses and graphs were completed initially using Microsoft Excel 2013 (Office 365) 2018 (Microsoft, Redmond, WA) and SPSS version 22.0 (IBM, New York, NY). Before and after analysis was completed in Sigma Plot 14.0 (Systat, San Jose, CA).

Results

From the 421 surfaces sampled with initial duplicate samples, 288/421 (68.5 %) both swabs demonstrated a result of <100 RLU and these surfaces were classified as “clean”. For 57/421 (13.5 %) of the tested surfaces, both swabs returned results >100 RLU. These surfaces were classified as “dirty”. For 18 % of surfaces (76/421), at least one of the duplicate swabs returned a result of >100 RLU and the other swab <100 RLU. These surfaces were classified as “equivocal”. The summary table of results is shown in Table 1 below.

The two swabs were plotted onto scatterplot graph (see Fig. 1 below). The cleanliness cut-off of 100 RLU is indicated on each axis thus dividing the figure into four quadrants. The use of colour in a heat maps based approach is recommended in healthcare applications over standard dot-mapped graphics [22].

The average of the initial two swabs are plotted in Fig. 2. For most surfaces, the results fall towards the clean end of the RLU testing spectrum with a long tail of surfaces with higher average RLU scores. When the “clean” group is assessed on the basis of surfaces with RLU levels below the level of 25 RLU the data remains skewed as shown in Fig. 3 below.

For the clean group of surfaces where the CIS was conducted (N = 139) comparisons of the first and second swabs were conducted. For those surfaces (N = 31) where both the swabs showed ATP results <25 RLU, there was no difference shown (P = 0.819, Wilcoxon). For the remainder of this clean group (N = 108/270), the difference between

Table 1 Swab data summary with before and after analysis with CIS.

	N	no CIS	CIS	Initial RLU	CIS Final	B4 & After
Clean (2 x < 100RLU)	288	149	139	34 RLU	6 RLU	P=<0.001 ^a
		<i>Clean 2x < 25RLU</i>	31	6 RLU	5 RLU	P = 0.29 ^a
		<i>Clean other</i>	108	43 RLU	6 RLU	P=<0.001 ^b
Dirty (2 x > 100RLU)	57	—	57	544 RLU	7 RLU	P=<0.001 ^b
Equivocal (1 x < 100RLU) + (1 x > 100RLU)	76	2	74	149 RLU	6 RLU	P=<0.001 ^b
Total	421	151	270			

CIS = Cleaning Intervention Step; RLU = Relative Light Units.

^a Paired T-test

^b Normality test (Shapiro–Wilk) failure: used Wilcoxon Signed Rank Test.

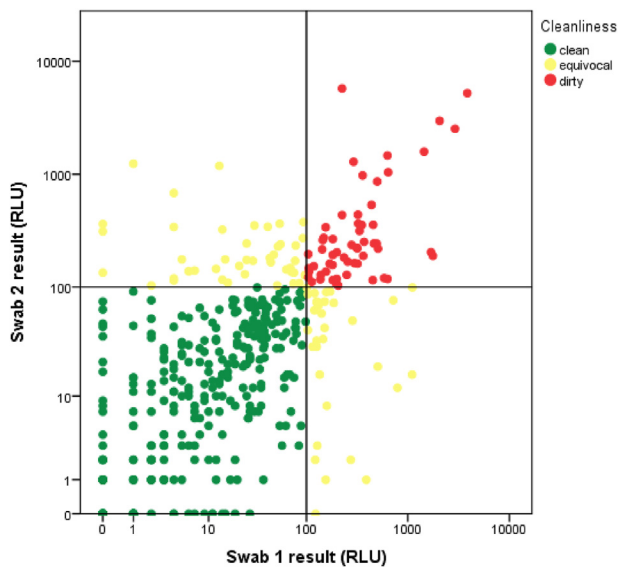


Figure 1 X–Y plot of all 1st and 2nd ATP swabs (duplicate locations).

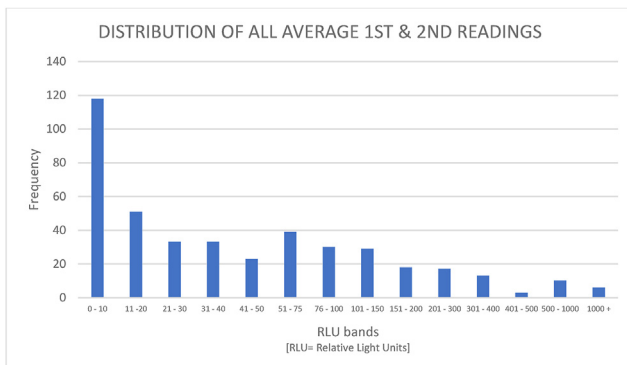


Figure 2 Distribution of all readings (average 1st and 2nd swab).

the groups (Swab 1 versus Swab 2), is highly significant ($P = <0.001$, Wilcoxon).

The duplicate swab introduces a new classification group of “equivocal” results which were present for 18 % of surfaces tested (76/421). The details on the equivocal group of results is set out in Table 2 below. Of particular interest are the 44/421 surfaces tested (10 %) where the first swab result was <100 RLU. This group can be treated as “false negatives”. Of these 44 surfaces, there are 11 results where the difference between the first swab and the second swab <100 RLU. This is consistent with the known inherent variance. On the other hand, the 33/421 surfaces where the difference between the swabs is > 100 RLU, could be reasonably assumed to represent variations in the cleanliness level of the surface (spots of uncleanness).

For the surfaces with a completed CIS the results of the average of the first two readings were compared to the final result of the CIS and this is shown in Fig. 4 below. There were 31/270 where the ATP results of the average readings

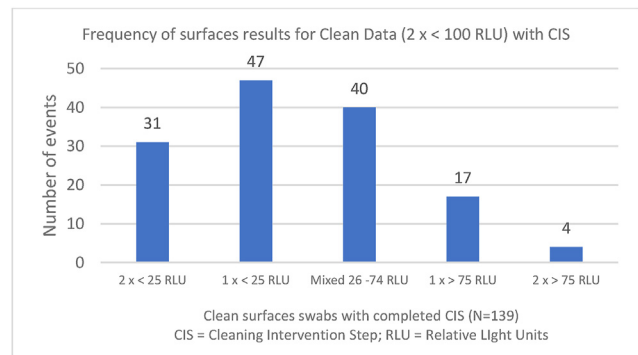


Figure 3 Distribution of swab results for clean surfaces (both swabs < 100 RLU) where CIS was completed ($N = 139$).

before the CIS, and the CIS results were all <25 RLU. For this small group there was no significant difference between the before and after results of the CIS ($P = 0.29$, Paired T Test). For the remainder there is statistically significant evidence ($P = <0.001$, Wilcoxon Rank Sum Test) RLU levels were improved through the additional cleaning.

An important finding was that for 7.4 % of surfaces tested with the CIS (20/270), the first cleaning wipe result did not achieve the goal of <25 RLU and additional CIS intervention was required. These results indicated a residual level of soiling and in four instances difficulty in achieving the required level of cleanliness where more than four separate CIS steps were required to achieve the <25 RLU goal.

An graphical outline of the simplified ATP algorithm is provided in Fig. 5.

Discussion

Despite the early promise, the use of ATP testing for cleanliness monitoring method has suffered criticisms and an inconsistent uptake. The initial claims for the universal usefulness of ATP testing were protested as somewhat overstated [3,5,23]. The second major shortcoming was unreliability due to inherent variability in the ATP testing devices and consumables [9–11,24]. There is a residual concern over the lack of correlation with the presence or absence of microorganisms that may also be detected on the tested surface [25–28].

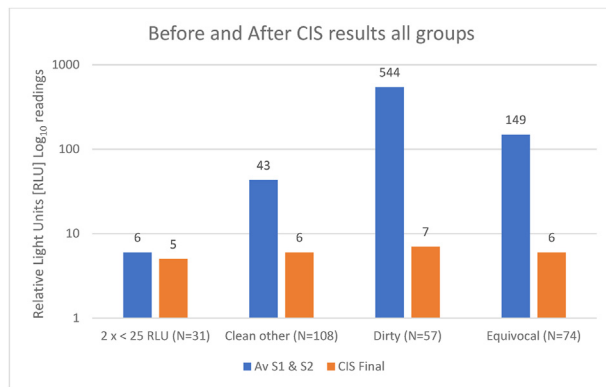
The ATP algorithm proposed a simple series of steps to untangle much of the risk of confounding information but required a lot of additional analysis and multiple cleanliness thresholds [4]. This new simplified method still uses duplicate samples but uses only two cleanliness thresholds (upper and lower cleanliness levels), which simplifies classification and analysis. The method has been simplified to just three effective steps as outlined in Fig. 5, which should reduce confusion in the practical application of the ATP algorithm to any chosen surface hygiene measurement.

The duplicate initial sampling both decreases the risks of sampling error, and also helps to identify inherent variability from true cleanliness concerns. The use of duplicate

Table 2 Equivocal Data ($1 \times < 100$ RLU) + ($1 \times > 100$ RLU).

	<100 RLU	>100 RLU	Average	Range	Median
1st swab	44 swabs	32 swabs	131 RLU	0–1110 RLU	76 RLU
% False Negative (44/421)	10 %				
Gap <100 RLU between 1st & 2nd	11/44				
Gap >100 RLU between 1st & 2nd	33/44				
2nd swab	32 swabs	44 swabs	164 RLU	0–1240 RLU	112 RLU
% False Negative (32/421)	8 %				
Gap <100 RLU between 1st & 2nd	16/32				
Gap >100 RLU between 1st & 2nd	16/32				
Total Equivocal Surfaces	76 surfaces (18 %)		149 RLU	0–1240 RLU	99 RLU
Total Surface Duplicates	421 surfaces				

RLU = Relative Light Units.

**Figure 4** Before and After CIS (Cleaning Intervention Step) results: all groups.

samples, of any size also reduces concerns around the size of the swab area (usually it is argued that the larger swab area will mitigate the risk of sampling error). This algorithm approach allows a rational sampling approach to irregularly shaped or smaller objects, devices or implements.

The assumption of any cleaning process is a level of improved cleanliness arising through the cleaning process, but timely feedback on a quantitative quality assurance measure has been lacking. ATP testing has been shown to improve cleanliness standards on healthcare surfaces where feedback from the testing is timely and continuous [29,30].

The data from this work demonstrates that without a duplicate swab cleanliness assessment tool such as the ATP testing algorithm, nearly one third (30.5 %) of surfaces harbour residual soil containing ATP above a nominal cleanliness threshold of 100 RLU (NB: this is a brand dependant figure using a Hygiena ATP testing device and consumables). Unfortunately, there is no universal definition on “clean”, although the process of cleaning has been best defined as “a process of removing unwanted soils” [31].

The cleaning intervention step (CIS) allows a defensible, scientifically credible and immediate investigation on the underlying basis of surface cleanliness. The CIS also uncovers difficult to clean or residually recalcitrant deposits of microbial soil to be removed, thus demonstrating that the original ATP result was real, and not an outlier [18,20].

Prior work has indicated that any two ATP testing results that are separated by an order of magnitude or greater represent real data and not just inherent variability [7,11]. So, separating the swab results of the equivocal group as shown in Table 2 above allows for a discrimination between likely inherent variance and real differences in the cleanliness outcome.

For 44 false negative results, 33 (44–11) surfaces could be reasonably regarded as “dirty” results on the basis that there is more than 100 RLU difference between the first and second swab (the first swab being < 100 RLU). This represents an overall false negative result of 8 % (33/421) which is alarmingly high. If a single swab approach is adopted, it is easy to see how these confounding outcomes would destroy the potential for correlations. For 33/421 surfaces within the equivocal group these surfaces can be treated as results probably belonging to the “dirty” classification. In either case, this equivocal group of results demonstrates the importance of a duplicate swab approach. From the equivocal group of results, 27/421 (11 + 16) surfaces had less than 100 RLU between the first and second swab result.

The cleaning intervention step (CIS) allows a scientifically credible investigation on the underlying basis of surface cleanliness, even following any normal cleaning process.

The opportunity to demonstrate improved cleanliness on 88 % of surfaces (239/270) is spectacularly confirmed by the CIS ($P = < 0.001$) with a simple aseptic wiping action.

The simplified ATP algorithm method mitigates many of the difficulties experienced with application of a single swab sampling method. The CIS demonstrates that soiling is not uniform on most surfaces.

One feature of the simplified ATP algorithm is the use of two cleanliness cut-offs. The first and higher reading for the Hygiena device was set at 100 RLU [21]. The second and lower threshold was set at 25 RLU which provided a level below which differences in results from surfaces were shown to be statistically indistinguishable [20].

The outcomes from these findings will require additional studies on a wider array of surfaces, medical devices, implements and other locations where cleanliness testing with ATP is conducted. One downside of this ATP algorithm approach is that multiple swabs (normally at least three) are used on each sample location. This increases the cost of

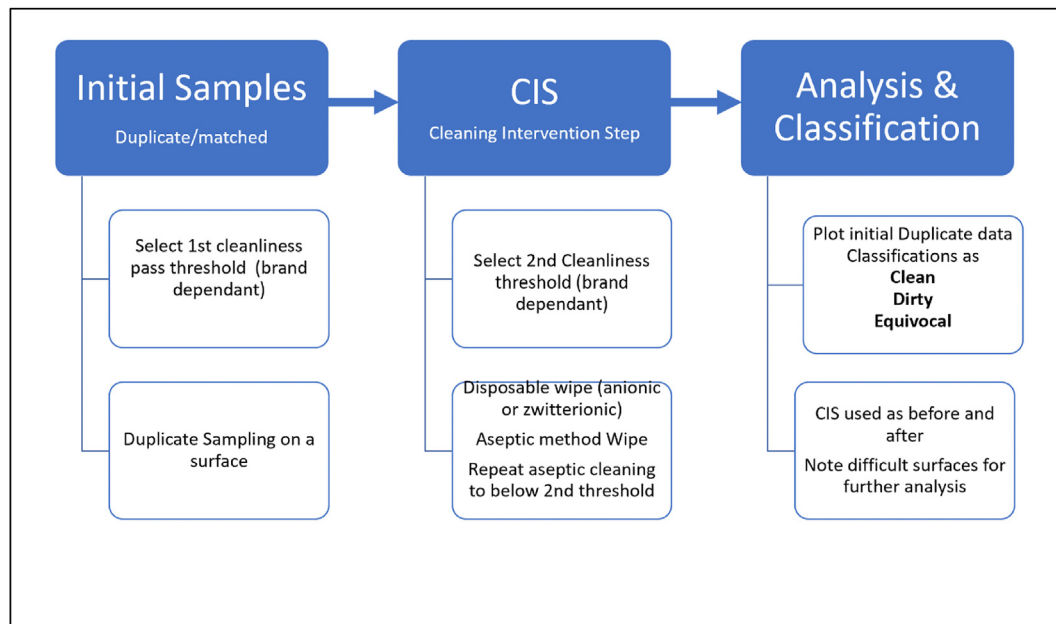


Figure 5 A simplified sampling & analysis process.

the monitoring modestly, and increases the time taken to achieve the data package, but the benefits achieved is significantly improved data reliability.

The use of this simplified ATP algorithm method does allow for the development of a traditional risk-based model to monitor cleanliness within healthcare or other settings based on the initial three level classification approach. This simplified ATP algorithm warrants further studies as a step towards improving data reliability and improved cleanliness standards for surface and re-useable medical device hygiene.

Ethics

No ethics approval was required for this study.

Authorship statement

The study was designed conducted and primarily authored by GSW. TOG assisted with study design, field sampling and manuscript development and corrections. PPF assisted in study design, statistical analysis & validation, corrections to the manuscript as well as the development of Fig. 1.

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Provenance and peer review

Not commissioned; externally peer reviewed.

Conflict of interest

There is no financial connection between any of the authors and with any supplier of ATP testing devices or consumables. GSW and TOG are employees of Whiteley Corporation.

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