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Research paper

A suggested sampling algorithm for use with ATP testing in cleanliness measurement

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Abstract *Introduction:* Rapid Adenosine Triphosphate (ATP) testing is becoming a commonly used method to measure cleanliness on reusable medical devices and healthcare environmental surfaces. Unfortunately, ATP testing devices suffer from inherent variability from a number of sources including imprecision in ATP measurement.
Method: This paper proposes a new sampling algorithm which reduces the impact of inherent variability and thus improves decision making when using ATP testing.
Conclusion: This algorithm can be applied across a variety of applications to provide more reliable data on measurable cleanliness standards for both medical devices and environmental surfaces. Formal trials using the new ATP sampling algorithm are required.

Highlights

- ATP testing is subject to inherent variability that causes imprecision with ATP readings.
- The variability problems undermine data validity from ATP testing.
- This algorithm mitigates the ATP testing variability and improves the reliability of ATP testing results.
- The algorithm can be applied across all brands of ATP testing devices.
- The algorithm improves the utility of ATP testing for all cleanliness monitoring applications.

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Introduction

The role of rapid ATP testing in cleanliness measurements

Cleanliness testing using rapid Adenosine Triphosphate (ATP) testing has been suggested as a quantitative method of assessing surface hygiene that is superior to simple visual inspection [1]. Rapid ATP testing devices are simple to use, lightweight and portable, provide an almost immediate reading and the consumables are relatively inexpensive. Rapid ATP testing devices measure all cellular ATP and not just microbial ATP, thus rapid ATP testing is a broad indicator of cleanliness from all biological soils and useful for cleanliness monitoring [2].

ATP testing is not a substitute for microbiological testing of surfaces although a number of studies have indicated the value of rapid ATP testing for cleanliness monitoring and training within healthcare settings [3,4]. The measurement of ATP contained in log scale dilution series of bacterial cultures does correlates with the concentrations of bacteria present and thus ATP testing provides an indication of bacterial contamination [5–7].

Studies using ATP testing for cleanliness measurements have compared various alternative methods for cleanliness monitoring and shown that ATP testing is a more reliable indicator [8]. Rapid ATP testing has also been used across a range of hospital settings to investigate cleaning performance standards [9]. ATP testing on a range of different healthcare environmental surfaces also showed that ATP was a sensitive method for setting cleanliness standards based on ATP measurements [10].

Studies using ATP testing to monitor the cleanliness of reusable medical devices such as endoscopes have also been published [11–13]. Establishing suitable cleanliness benchmarks using ATP testing has also been suggested [12,14]. The use of rapid ATP testing for cleanliness monitoring of Gastro Endoscopes (GES) was also compared favourably with monitoring of residual protein and microbial contaminants noting that the detection limits for differing methods were not always consistent or appropriately scaled [15].

It has been noted that the use of ATP testing for cleanliness monitoring within healthcare settings is a currently developing area of science that requires further study [16].

However, questions have been posed over the reliability of the results arising from ATP testing [17,18]. Recent national guidelines have chosen to not incorporate ATP testing into cleanliness monitoring for healthcare environmental surfaces [19].

Variability and imprecision with ATP testing

The first difficulty when using ATP testing devices in the field, is that the surfaces being tested may not be uniformly covered by human cells, bacterial cells or even food based cellular materials. The variability of soil distribution on a surface, whether bacterial or other ATP rich soils, is a confounder for ATP testing even before device related issues are considered. So, a low reading on one location may not indicate a low reading across entire surface.

This problem is not unique to ATP testing devices.

The sampling area for swabbing with ATP testing devices is also not standardised. When ATP testing was first proposed by Griffiths et al. the sampling area was a 10 × 10 cm (100 cm^2) area with a target level of cleanliness of 500 Relative Light Units (RLU) using a Cleantrace ATP testing device [1]. Following studies using the same branded equipment and same sampling area the cleanliness standard was reassessed and suggested as only 250 RLU [10]. More recently a study using a different brand of equipment (Hygiena) has suggested that the cleanliness standard be reduced to just 100 RLU using a 100 cm^2 sampling area [20]. Finally, another study using the Hygiena ATP reaffirmed the 100 RLU cleanliness threshold, but reduced the sampling area to a more practical 2 × 5 cm (10 cm^2) area [21].

Another complication is that every ATP testing device reads on a brand specific and arbitrary scale even though all share the common name of Relative Light Units or RLU [22]. The results of comparison testing of different brands of ATP testing devices has shown wide dissimilarities between the RLU scaling at standardised testing levels with both pure ATP and bacterial cultures [6,7].

An earlier study found that for some brands of ATP testing devices (e.g. Cleantrace [3M], Kikkoman), the lower level of detectability (LLD) is appreciably below the lower level of quantitation (LLQ). In plain language that means that those ATP devices can detect low levels ATP but cannot precisely or reliably indicate the quantity of ATP which is detected. At lower levels of ATP contamination, the meter readings contain relatively higher levels of error.

Where an ATP testing reading is obtained which is below the LLQ, then this data should be expressed as 'less than "***n***" RLU' (where "***n***" = the LLQ). For practical field usage, it would be better if the LLD = LLQ = 0 RLU on the ATP testing device. Unfortunately, this is not the current situation for all ATP testing devices and without a validation process, most field users would be unaware of these failure risks on the particular brand of ATP testing device [7]. The LLQ of several ATP testing devices is indicated in Table 1.

In addition to brand differences between ATP testing devices there is a problem with inherent variability. Precision is the ability of the ATP testing device to repeatedly provide the same RLU reading at the same ATP

Table 1 Suggested cleanliness thresholds and corresponding RLU for 3 ATP device brands.

	Cleanliness threshold	Hygiena	Cleantrace	Kikkoman
Initial cleanliness threshold	T_C1	100 RLU	500 RLU	460 RLU
Secondary cleanliness threshold	T_C2	50 RLU	250 RLU	230 RLU
Tertiary cleanliness threshold	T_C3	25 RLU	125 RLU	115 RLU
Lower Limit of Quantitation	LLQ	0 RLU	100 RLU	90 RLU

concentration. With ATP testing devices, the imprecision is high reflected by the Coefficient of variance (Cv) [18,23]. Controlled experiments have demonstrated that a Cv of 0.4 is a frequent occurrence [24]. In studies using a single sampling point, where the Cv is above 0.4, there is a 20% chance that any reading could be wrong by a factor of two [25].

The variability problems could be mitigated substantially with the development of a unified RLU scale against which the ATP testing devices could be calibrated. This would allow for accuracy and improved precision.

But short of re-engineering the ATP devices, the most practical way to mitigate this variability is through the use of a well-considered sampling plan [26]. We have previously trialled using the median of quadruplicate testing to remove outlier impacts [26]. This has the immediate drawback of additional costs in terms of both time and consumables.

In summary, we believe that the practical application of current ATP testing devices in healthcare settings requires standardised instructions or guidelines specifying the sampling area, sampling methodology and number of samples. We propose the following algorithm as a first attempt to carefully and systematically address these issues.

Methods

The ATP testing algorithm

The goal of this algorithm is to maximise confidence in the practical application of ATP testing devices in the measurement of ATP. This sampling algorithm can improve the reliability from the ATP readings to more than 99% certainty based on known probabilities. Central to the use of ATP testing as a monitoring process is a carefully constructed sampling plan including an appropriate array of high touch objects and surfaces, relevant for targeted cleaning and therefore requiring a higher standard of cleanliness monitoring [20].

There are four steps in this algorithm.

1. A standardised ATP swab sampling area of $2 \times 5\text{cm} = 10\ \text{cm}^2$.
2. An initial two sample approach, using adjacent sampling on the surface to be tested. Additional samples may be required where the initial duplicate sample results are ambiguous. On objects with small surface areas the duplicate sampling approach will necessitate overlapping samples.
3. A four-tiered cleanliness rating (Table 2) based on the known dynamic range and existing published test results of the ATP testing devices used for sampling.
4. The final control step for cleanliness verification using a disposable wipe, and then re-testing (single ATP swab) of the cleaned area.

Initial ATP sampling in duplicate

If the 'true' ATP value for a surface was exactly equal to the cleanliness threshold — let's say 100 RLU — then the random variation in the measurement device could be expected to result in about half of the individual readings being above and half being below this true value. So, a single measurement interpreted against the 100 RLU threshold has a 50% probability measuring less than the cleanliness threshold (a false negative result).

Table 2 Suggested formula for cleanliness standard setting with any ATP testing device.

Identified cleanliness threshold	Defining the cleanliness thresholds relationships
T_C1	$T_C1 = T_C2 \times 2 = T_C3 \times 4$
T_C2	$T_C2 = T_C1 \div 2 = T_C3 \times 2$
T_C3	$T_C3 = \text{LLQ} + 25$ RLU (may be higher for some devices)

We seek to improve decision making by combining two or more ATP measures. In the above scenario where the true ATP is exactly equal to the threshold, there is only 25% (50 % × 50 % = 25%) of two readings measuring below the threshold and 12.5% chance for 3 readings in a row (6.25% for four readings in a row). Where the true ATP values are higher than the threshold, the probability of false negative readings decrease rapidly.

As discussed above, our previous research has suggested that a Cv of 0.2 is average for any set of readings. A Cv as high as 0.4 will also occur in normal field usage. Based on the work of Reed et al. a Cv of 0.4 suggests that any two measures from the identical surface could differ by a factor of 1.5 or more in nearly half of all trials and differ by a factor of 2 or more in 1 in every 5 trials [23]. In such cases, a reading of 50 RLU and 100 RLU could be generated from the same surface cleanliness and still represent the same result [24,25]. While a Cv of 0.4 was relatively infrequent, our proposed algorithm is conservative and based on the possible two-fold difference in RLU measures. Within the health care environment, the emphasis should be to minimise false negative readings i.e. to minimise the chance of mislabelling a dirty surface as a clean surface.

Combining the above considerations and using the 100 RLU cleanliness threshold for convenience we can conclude:

- Two readings below 100 provide good evidence of cleanliness (at very worst 0.25 risk of false negative result if both readings are very close to the threshold)
- Two readings below 100, one of which is below 50 provides strong evidence of cleanliness (given the Cv of 0.4 is relatively rare, the possibility of one of the observations lying two-fold below the threshold is very small)
- Two readings below 50 providing very strong evidence of cleanliness
- Two readings below 25 providing exceptional evidence of cleanliness.

By the same logic, two readings above the cleanliness threshold should be treated as insufficiently clean. The more difficult to interpret results is one reading below and the other above the cleanliness threshold. In such cases we recommend a third swab with the median of the three to be compared against the cleanliness threshold.

The three-tier cleanliness threshold

The four groups are separated by the three cleanliness thresholds, being T_C1, T_C2 and T_C3. These are shown in Table 1 below with the suggested RLU readings for three tested ATP devices.

For at least two devices (Cleantrace and Hygiena) cleanliness thresholds have been proposed. The upper cleanliness threshold for the Hygiena device is indicated as 100 RLU [20,21]. For the Cleantrace device the first cleanliness threshold was firstly suggested at 500 RLU [1]. More recent work revised this down to 250 RLU [10]. For the purposes of this algorithm, and given what is now known about the variance issues, the first threshold of 500 RLU for the Cleantrace ATP device is accepted as appropriate for T_C1 and the revised threshold conforms to the expectations for T_C2.

It is readily acknowledged that other branded ATP testing devices could be incorporated into our algorithm subject to presentation of the dynamic range and establishment of the LLQ for that device.

The relationship between the three tiers of cleanliness threshold is outlined in Table 2. Using the two-fold difference rule, the initial cleanliness threshold (T_C1) will be twice as much as the secondary threshold (T_C2) and four times the tertiary or lower cleanliness threshold (T_C3). The lowest cleanliness threshold should be set just above the LLQ for the individual brand of ATP testing device, and 25 RLU is suggested as a guideline for the reading gap between the LLQ and the T_C3 threshold.

Two readings above T_C1 are interpreted as strong evidence for insufficient cleanliness. Two readings which are below T_C2 are interpreted as very strong evidence of cleanliness and two observations below T_C3 are interpreted as very strong evidence of cleanliness.

The sampling process steps for ATP testing

The process of ATP testing (sampling) follows the protocol summarised in Fig. 1. Firstly duplicate samples are taken using the ATP testing device based on a standardised sampling area of 10 cm^2. The ATP swabs are taken on immediately parallel locations on the chosen environmental surface or medical device. Wherever possible the duplicate sampling areas should not be overlapped. Whilst it is preferable never to overlap sampling, on small surfaces (e.g. a four pointed tap handle) this may be problematic so overlapping of any sampling should be recorded.

From the duplicate sampling four possible outcomes are obtained. Firstly, both ATP testing results, measured in RLU, may be below the initial cleanliness threshold (T_C1). These results are classified as group A in the algorithm shown in Fig. 2. Secondly, both results could be RLU readings above T_C1, and these results are shown as group B in the algorithm. For both groups A & B, the median RLU result is accepted. In either case, a cleanliness verification step is then conducted.

The third possible outcome from the initial duplicate testing (group C) is that the two results may indicate RLU readings on either side of T_C1. In this case a triplicate reading is required. If one of the three readings has a value more than ten-fold different from any other value in the sampling group, then a fourth sample reading is also required. Each additional ATP sample should be taken by swabbing on the same surface and if possible the sampling areas should not be overlapped.

For any surface sample group of readings which requires triplicate or quadruplicate ATP samples these samples are classified as sample group C on the algorithm. The median value is accepted as the indicative value to group C samples.

This ATP sampling algorithm does not intend to overcome what are sampling methodology issues. The non-uniform distribution of ATP rich soils can be expected to interfere with results patterns. For this reason it is important to sample from a sufficiently large variety of surfaces or implements to ensure that cleanliness estimates are less likely to result in either false positive or false negative conclusions.

After the last sample swab is taken, the surface may also be sampled for specific microbial presence. Where environmental sampling to be conducted, it is recommended to use a more aggressive environmental sampling method on a parallel surface and not overlapped with the ATP sampling regions [27].

The cleanliness verification step

The cleanliness verification is required to test the cleanliness capacity of the surface as well as to provide another

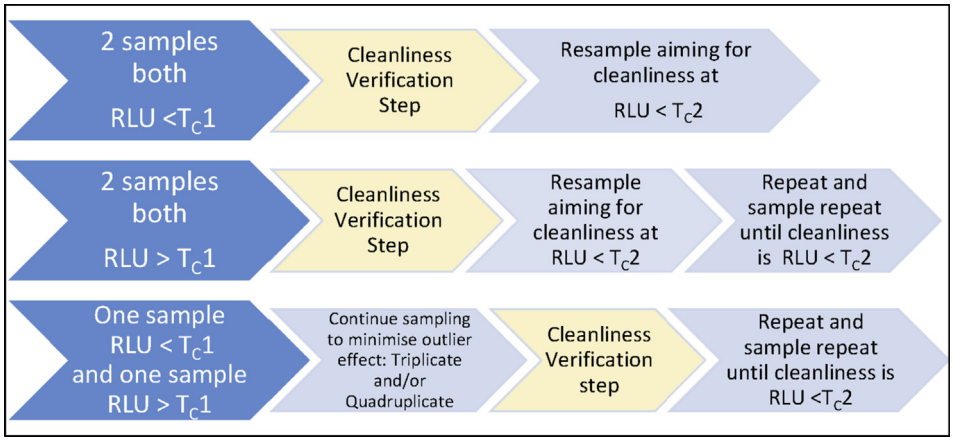

Figure 1 ATP testing algorithm as a flow chart using cleanliness thresholds (see Tables 1 and 2).

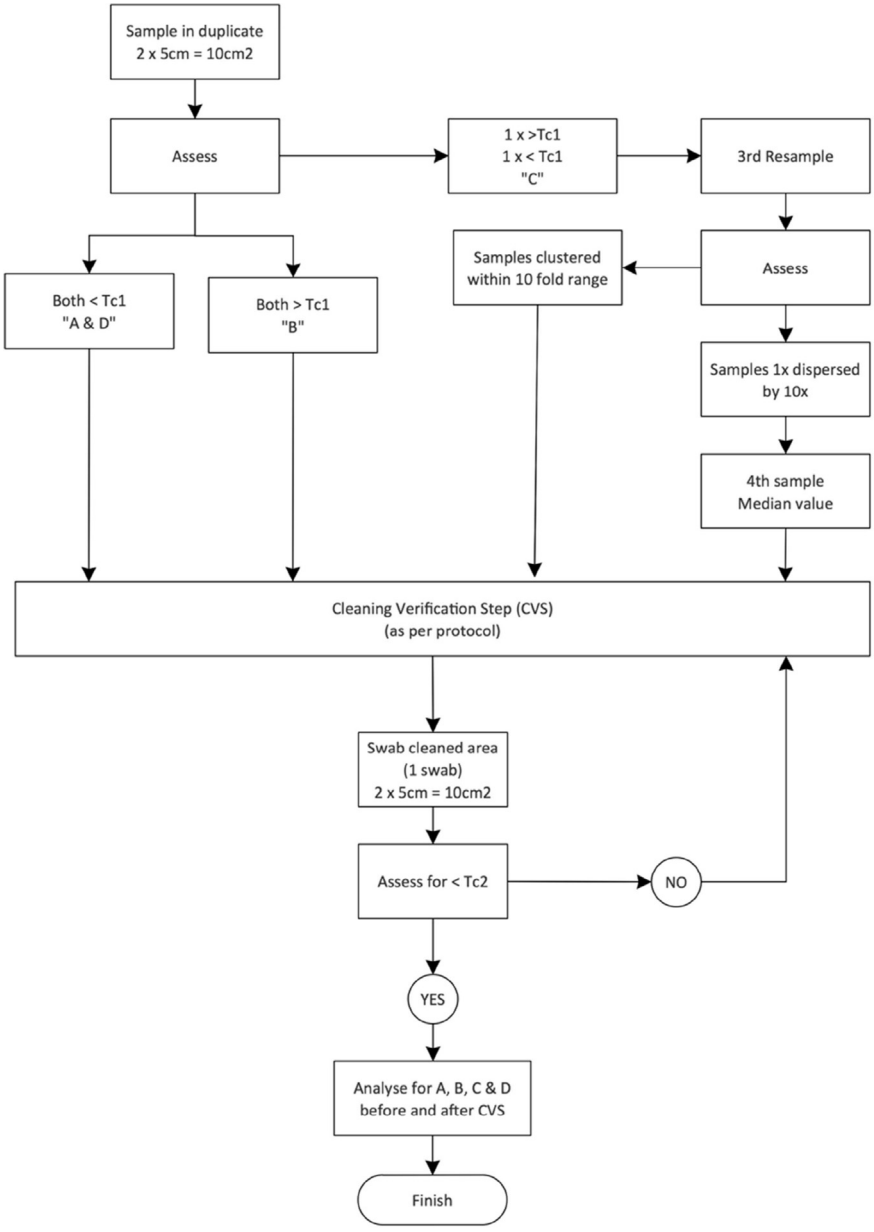

Figure 2 The ATP sampling algorithm.

level of certainty over the initial sampling. The cleanliness verification step is undertaken immediately following the initial testing and prior to any further use of the surface or medical device. Thus all of the sampling is finalised quickly and the surface or device returned to normal uses in a time efficient manner.

The cleanliness verification step is conducted using a disposable neutral or anionic detergent wipe. The wipe is used as per Maillard et al. with a single wipe used on a single surface and wiped in a single direction [28]. In practice this is done by removing the wipe from the packet with gloved hand, and using the first side (side 1) to wipe generally over the device or surface being tested to thoroughly wet the surface. The wipe is then folded in half so that side 1 is on the inside of the folded wipe. The wipe now has two surfaces on side 2, being 2a and 2b. The area being tested is then strongly wiped using the 2a side of the wipe in a single wiping action across the surface or device.

The wipe is then turned over so that side 2b is facing down, and this side is then also wiped strongly across the surface in a single direction. Thus a double wiping action ensures cleaning to the equivalence of a disinfectant applied to the same surface [29]. Care should be taken when wiping to keep the wiping action inside the wetted area so that any contamination outside of the wetted area is not introduced into the testing area.

The cleaned area is allowed to air dry for several moments. A final ATP swab test is immediately conducted once the cleaned area is fully dry and before any subsequent hand or other contact. The results of the cleanliness verification step, second stage ATP reading should demonstrate a reading below the secondary cleanliness threshold (T_C2).

This secondary threshold is set at half of the RLU reading from the initial cleanliness threshold. Where the second stage ATP reading is above T_C2, then the cleanliness verification step should be repeated.

Our initial work on this step has indicated that with some very unclean surfaces, the cleanliness verification step may be required to be repeated several times to bring the cleanliness level into compliance with T_C2. This secondary threshold is set a level outside of the two fold variance level from the initial threshold.

Initial field trials have indicated that samples designated as Group D represent surfaces where no statistically significant improvement can be achieved via the cleanliness verification step.

The full testing algorithm is outlined in Fig. 2.

Discussion

This algorithm is designed to reduce the impact of inherent variability and imprecision on any individual sampling surface or medical device. The algorithm makes use of previously reported probabilities and, based on the known variance of ATP testing devices, uses a multiple sampling step to increase certainty over the true values obtained when using an ATP testing device. The use of the cleanliness verification step further confirms the initial reading results by demonstrating the cleanliness value that would be possible with a validated cleaning method.

Our work using this algorithm has indicated that a tertiary threshold which is half of the secondary threshold will be required to demonstrate a level of cleanliness which cannot be improved upon. In this algorithm the upper threshold acts as a true upper threshold when the key secondary threshold is 50% of that value. Thus, any variance within the expected range of a factor of 2 from the key secondary threshold will fall within the nominal range of the upper and the lower thresholds.

This simple approach is applicable to the three ATP testing devices so far validated for dynamic range and variability. Other ATP testing devices will require a clearly defined LLQ before the algorithm may be used with the cleanliness thresholds as set out in this paper.

So, for Hygiena ATP devices the key threshold is set at 50 RLU, thus rendering 100 RLU as the uppermost threshold whilst preserving values of 25 RLU and below as the lower cleanliness value. The LLQ has been demonstrated at 0 RLU for the Hygiena device. For the Cleantrace device a tiered cleanliness threshold is suggested with the upper threshold being 500 RLU, the secondary threshold as 250 RLU and the lower cleanliness threshold as 125 RLU. The LLQ for the Cleantrace device is shown to be around 100 RLU. The LLQ for the Kikkoman device is shown to be around 90 RLU.

The testing for this algorithm was initially conducted within a food surveillance context where cleanliness measurements are also visually based and are in urgent need of a more scientific approach. Work applying this algorithm within a healthcare cleanliness context has commenced with several Australian studies using the algorithm sampling methodology as outlined. More work is required to validate this method within healthcare settings and with a greater variety of branded ATP testing devices.

There are a number of limitations with this algorithm. Firstly, the LLQ values for ATP testing devices is limited to just three brands where data is known and published. Secondly, field testing of the algorithm remains limited at this time. Thirdly, the work is proposed without any support from any of the ATP testing device manufacturers. Finally, there remains no direct link between detectable levels of ATP and the presence or absence of an MRO. So whilst ATP testing can reliably detect general cellular cleanliness, the level of ATP detected is not a direct indicator of disease or infection risk.

Whereas the broader sampling issues such as soiling distribution and types of soils are subject to the normal vagaries' of sampling methodology, and are not overcome through this algorithm, the use of the algorithm does improve the value of a well-designed sampling approach to improve the overall reliability of cleanliness monitoring within healthcare settings.

We propose this algorithm as an improvement to the science behind cleanliness testing in healthcare settings using ATP testing devices. This algorithm would be more useful in the context of a standardised RLU scale, but this is not currently available. Advances in the standard of consumables and reduction of inherent variability in ATP detection would enhance the usefulness of this Algorithm and ATP testing as an infection prevention or hygiene measurement tool. Studies using ATP testing devices have demonstrated the advantage of speed of response for cleanliness measurement, and this algorithm is intended to improve reliability in addition to timeliness in answering questions over surface or medical device cleaning.

Conclusion

It is important that there is a reliable and quantitative method for cleanliness monitoring in situations where visual inspection is the only existing alternative for real time assessment and feedback on surface hygiene. This new ATP sampling algorithm provides a superior level of certainty with field based ATP data through the mitigation of inherent device imprecision and variability. The sampling algorithm requires structured field testing to confirm its practical use in a range of different settings. The use of ATP testing for improvement the quality monitoring of surface cleanliness and cleaning outcomes may be improved through the application of this new approach.

Ethics

No ethics approval was required for this study.

Authorship

Authorship by all three authors is confirmed in alignment with journal policy. All three authors actively contributed to both the research and the writing of the paper for submission to the journal.

Conflicts of interest

None of the authors have any commercial or financial arrangements with any manufacturer or brand of ATP testing device.

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