A study into the validation of ATP testing devices for integrated cleanliness monitoring within healthcare settings

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A thesis submitted in fulfilment
of the requirements for the degree of
Doctor of Philosophy (PhD)

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Statement of Authentication

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I hereby certify that the work presented in this thesis in fulfilment of the requirements for the degree of doctor of philosophy (PhD) is to the best of my knowledge, original except for those parts as acknowledged in the text. I also certify that the material has not been submitted either in part or in full for any other degree, whether enrolled at this university or any other institution and I certify that I have complied in all other aspects with the rules, requirements, procedures and policies relating to the award of this Degree at the Western Sydney University.

Gregory Stuart Whiteley

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And that thanks extends onto the others in my family including Jemima, Mitchell and Holly, the late Rusty and also to Mimi. And to both my Mum and Dad, who have always believed in me. A belated thanks also to the Late Dr Margaret Sheldon OAM, whom I know would be so proud and pleased for me and the entire family group at finishing this PhD.

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AMDG

Greg S Whiteley

Preface

This completed document is submitted to Western Sydney University (WSU) in fulfilment of the requirements of the Doctor of Philosophy (PhD) degree as a series of publications and overarching statement. My initial enrolment in the higher degree program was in the first semester of 2008. The project consisted in an initial literature review followed by a Confirmation of Candidature in 2010.

Shortly after the confirmation of the project, the research phase commenced and included laboratory studies, a risk assessment study, five poster presentations, five publications arising from the research including a field based pilot study which was described in the fifth publication, several letters to the editor canvassing aspects of the research, and finally the compilation and presentation of this thesis.

The requirements for awarding of a PhD by publication must include at a minimum four, full length, scholarly and peer reviewed publications and an overarching statement as a thesis. This PhD thesis includes five scholarly and peer reviewed publications as the second publication was published as a 'research brief', albeit with full peer review and within a special edition on environmental hygiene through the journal Infection Control & Hospital Epidemiology.

The format of this thesis generally follows the WSU guidelines found in the Higher Degree Research Examination Handbook (http://www.uws.edu.au/__data/assets/pdf_file/0019/70822/ORS3474_HDR_Handbook_Exams_booklet_A5_LR3.pdf:doi 21st January 2016).

There are a number of minor exceptions from the guidelines within this thesis. Firstly the order of inclusions is slightly varied to take account of the content, which is for a PhD thesis as a series of publications. Secondly, the references appear at the end of each discreet chapter, both in the compilation and each of the published references, published letters, and posters. At the start of each of the first four chapters is a short precis which takes the place of an abstract for each chapter. This precis outlines in a simple form the content to be appreciated through the following material.

The page numbering has been applied as per the WSU guidelines, excepting that the five publications have retained their pagination as published. The page numbers applied to the section headings have been kept in the proper sequence throughout the thesis.

I hope that you enjoy reading this account of the study and the published outputs arising from this body of original work.

Greg S Whiteley

1st August 2016

List of Abbreviations

Abbreviation	Full form or title
ABHR	Alcohol based hand rub
AJIC	American Journal of Infection Control (Journal of)
ASID	Australian Society of Infectious Diseases
ATCC	American type culture collection
ATP	Adenosine triphosphate
Cv	Coefficient of variance
E coli	*Escherichia coli*
FM	Fluorescent marker
FMEA	Failure mode effects analysis
HAI	Healthcare associated infections
HCW	Health care workers
HI	Healthcare Infection (Journal of)
HIS	Hospital Infection Society
HTO	High touch object (or surface)
HPLC	High Power Liquid Chromatography device
ICHE	Infection Control and Hospital Epidemiology (Journal of)
ICU	Intensive care unit
IFIC	International Federation of Infection Control
LCMS	Liquid Chromatography Mass Spectrophotometry
MRO	Multi drug resistant organisms
MRSA	Methicillin resistant *Staphylococcus aureus*
NHMRC	National Health and Medical Research Council
NSW	New South Wales
P aeruginosa	*Pseudomonas aeruginosa*
RLU	Relative light units
ROC	Receiver operating curve
SHEA	Society for Healthcare Epidemiology of America
S. epidermidis	*Staphylococcus epidermidis*
TGA	Therapeutic Goods Administration
The 5 moments	The 5 Moments of Hand Hygiene campaign
TSB	Triptone soy broth
VRE	Vancomycin resistant *Enterococcus* (*faecium* or *faecalis*)

Glossary of Terms

Term used	Definition for this thesis
Biofilm	A group of bacteria which are joined together in a material that has been produced by bacteria, and which provides a sustainable framework for bacterial survival, growth and dissemination
Cleaning	The process of removal of soils including unwanted microorganisms from a surface
Colonisation	The presence of a microorganism on the skin or other body cavity of a patient or health care worker
Disinfectant	A product which kills a defined number of microorganisms and which is registered in Australia as either: i. Disinfectant – Hospital Grade; ii. Disinfectant – Instrument Grade or iii. Sterilant; under the Therapeutic Goods Order 54, 1996 (as amended) which provides the Australian regulatory framework for Disinfectants and Sterilants
Hazard	Anything which has the potential to cause or result in harm to an individual or environment
Health Care Worker	A doctor, nurse or other person employed and working within a health care setting and who is performing duties pertaining to patient health or maintenance of the hospital or health care environment.
Hygienic	A state of cleanliness of a surface which has no hazards present
Infection	A patient status where a microorganism has begun to grow inside a normally sterile area of a person, and which growth has triggered an immunological response within that person
Risk	The probability of a change in status, either adverse or positive in nature
Risk Assessment	The detailed analysis of the risk of different outcomes arising from a process or of processes (such as cleaning or cleaning monitoring)
Sanitising	The process of removing microorganisms sufficiently well to be considered hygienic
Soil	Foreign matter on a surface which may or may not include microorganisms
Surface	The outside of an object, implement, wall, desk or other piece of furniture, or any other item used within a healthcare setting

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A study into the validation of ATP testing devices for integrated cleanliness monitoring within healthcare settings

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Abstract

There is a problem in hospital cleaning. There is currently no scientific monitoring method that provides timely and reliable assurance that harmful bacteria have been removed by the cleaning processes.

This PhD investigates the validation of commercially available testing devices intended for the rapid detection of adenosine triphosphate (ATP), which may provide a quantitative surrogate estimate of surface cleanliness based on ATP measurements. The findings indicate that ATP testing devices, despite problems with scaling and imprecision, provide immediacy in readings which enables the incorporation of ATP testing into an improved and integrated cleanliness monitoring process for applications within healthcare settings.

Introduction

There is a problem in hospital cleaning. The problem is that there is no accepted and credible scientific measurement of the outcome resulting from the cleaning process. A monitoring approach is required that can reliably assess both the efficacy and efficiency of hospital cleaning and the standard of cleanliness of healthcare surfaces.

There are two existing methods which are used to verify the cleaning and both are inadequate. The most common method of cleaning monitoring is visual inspection, which whilst it is quick and easy, is not overly scientific. Visual inspection in isolation cannot assess if the cleaning process has occurred, nor the time since the cleaning process on a particular surface was completed and the visual appearance does not correlate with actual surface cleanliness using microbiological methods.[1]

The other existing method of cleaning monitoring is environmental or surface microbiology which takes too long between sampling and obtaining results, is expensive, and has sampling difficulties.[2]

There are two new methods proposed to measure and monitor cleanliness.

The first new monitoring method utilises hand held devices that rapidly measure the presence of adenosine triphosphate (ATP). ATP is present in all living cells and the presence of ATP is proposed as a proxy indicator of surface soiling with biological based materials.[1]

The second new cleaning monitoring method uses a fluorescent mark (FM) to assess the efficiency of a cleaning process an environmental surface (FM spot removal).[3]

Both of the new methods provide timely information for monitoring purposes and both may be integrated into cleanliness monitoring processes for hospital applications.[4]

This PhD study investigates the validation of several commercially available, rapid ATP testing devices, which can be used to provide a quantitative estimate of surface cleanliness based on their ATP measurements. Rapid ATP testing devices make use of the 'firefly reaction' to generate and measure a luminescent reaction which is loosely proportionate to the level of cellular soiling present on a surface (ATP + Luciferase = Light). This light response is expressed in a scale known as Relative Light Units (RLU).

The results from the studies in this PhD are published in the five included published papers. These findings indicate that rapid ATP testing, despite imprecision and scaling difficulties, can be included in an integrated monitoring processes, albeit with careful consideration over sampling methodology and the interpretation of the data/readings arising from the use of rapid ATP devices.

The four chapters which follow this introduction are the overarching statement that explains the examinable work. The first chapter outlines the evolution of the project; the second chapter reviews the methods used in the five papers; the third chapter provides a detailed discussion over the context and implications arising from each of the papers, and finally a fourth and concluding chapter summarises the key findings.

The five published papers each examines a different aspect of the overall validation and integration of rapid ATP testing into a cleanliness monitoring process. The five papers are included after the first four chapters, and are described briefly as follows.

The first paper outlines the findings of a series of studies of three commercially available ATP testing devices, to describe and document the dynamic range, upper and lower limits of detection, linearity of response and the precision of response of each ATP device. The accuracy of response which would normally be included in validation studies could not be conducted due entirely to the relative and idiosyncratic scaling used by each branded ATP device. A novel method of direct application of pure ATP was developed to establish device validation without interfering with the individual dose responses of each commercial device. The findings of this paper indicate the key features of each ATP device against the core validation criteria.

The second paper (which is a short 'Research Brief'), applied the findings from the first laboratory study and, using the statistical measure of Coefficient of variance (Cv), presented graphical information on the uncontrolled variability of the ATP devices. The measurement of Cv is obtained by dividing the mean into the standard deviation for each data set and allows dis-contiguous data sets to be normalised for comparative purposes. The results demonstrated that when challenged using pure ATP, all of the ATP testing devices displayed a Cv above 0.4. This was surprisingly high when compared to the calibrated High Performance Liquid Chromatography (HPLC) results.

The third paper in chronological sequence considered the findings arising from the laboratory studies to consider the risks arising from integrating ATP testing into a cleanliness monitoring program. The paper used a risk assessment tool known as Failure Mode Effects Analysis (FMEA) to assess each of the cleaning monitoring methods (visual inspection, surface microbiology, ATP testing and FM). The findings indicated that by combining the four cleanliness monitoring methods into a single integrated monitoring process, the strengths of each method could be used to mitigate the weaknesses from each of the other monitoring methods. This finding is presented as a novel and new approach for integrating the cleanliness monitoring for use in healthcare settings.

The fourth paper (in the chronological sequence of publication) applies the results from the first and second studies and extends this analysis to include the use of bacterial species in addition to pure ATP. This provided a more comprehensive measure of the precision of the dose-response for each of the ATP testing devices. The variability of the dose-response was again examined using Cv, which is fully described in the paper. The experiments included a fourth ATP device and results demonstrated that the Cv (variability) for each of the tested ATP devices was in excess of Cv > 0.4. The high variability suggests that any reading taken on any of the four ATP devices has a 20% chance of being wrong by a factor of 2. This finding has important sampling and interpretative implications for those using rapid ATP testing devices in cleanliness monitoring.

The fifth and final paper applied knowledge obtained during the earlier published studies and applied this learning into a field based pilot study investigating an integrated cleanliness monitoring approach. This paper was selected as the lead article in the December 2015 issue of the American Journal of Infection Control. There were a number of key findings, and importantly the use of rapid ATP testing applied within an integrated cleanliness monitoring process was shown to be significantly better than using a traditional, one dimensional, list based sampling approach.

This PhD is the accumulation of a single and contiguous group of studies which examines the validation and performance of ATP testing devices. This PhD further investigates the incorporation of rapid ATP testing devices within an integrated cleanliness monitoring process for healthcare settings.

The results demonstrated in this PhD underpin an enhanced scientific platform for on-going research to further improve the reliability of cleanliness measurements within healthcare settings. This thesis is submitted for the satisfaction of requirements for the awarding of a Doctor of Philosophy Degree.

Goals for the study

In the Confirmation of Candidature (March 2010), the overarching aim of the research was to:

"*…investigate an integrated approach to cleanliness monitoring, relying on modern scientific methods… that reflects a truly validated standard of cleanliness at a sub-visual level. An accurate appreciation of actual surface hygiene and cleanliness can then more accurately inform infection control practice. By demonstrating that interpretation and validation of cleaning in health care be improved this will remove a critical current confounding factor (validity of cleaning processes) that contributes to the difficulty in preventing the spread of HAI within the health care setting*."

The initial research goals of the project were to:

1. Conduct a series of validation studies of the new cleanliness monitoring methods of ATP testing and FM spots to confirm their scientific validity for field use; and
2. Compare the ATP and FM methods with visual inspection and microbial recovery so as to develop an integrated approach to cleanliness monitoring which is scientifically valid; and
3. Complete a field based cleanliness monitoring study using an integrated model within a healthcare setting to demonstrate the value of improved scientific measures of cleanliness monitoring for use in practical infection control.

Following the initial laboratory studies it became clear that the FM approach measured cleaning efficiency (i.e. the physical effort of wiping a surface – a qualitative factor) and did so with robustness. Validation studies published by Carling and others established FM as a reliable measure of cleaning effort and efficiency.

However, ATP testing systems measure the output of cleaning effort through assessment of the quantitative cleanliness level achieved through the cleaning process (a quantitative factor). Unfortunately, it became quickly apparent that ATP testing systems required substantially greater laboratory experimentation in an effort to achieve a validated status upon which ATP testing could be used reliably for cleanliness monitoring within healthcare settings.

The confirmed goals for the research project were to:

1. Investigate the validation of ATP testing systems for reliable cleanliness monitoring; and,
2. Investigate the four major cleanliness monitoring methods (ATP, FM, visual inspection and microbial recovery) to provide a reliable and integrated method of cleanliness monitoring within healthcare settings: and,
3. Investigate the application of an integrated cleanliness monitoring process for practical infection prevention within a healthcare setting.

The hypothesis being tested is:

“ATP testing is a valid and reliable method of cleanliness monitoring which can be incorporated into an integrated cleanliness monitoring methodology for use within healthcare settings to improve practical infection control”.

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Chapter 1

The evolution of the research project

Précis

This PhD project was commenced in 2008 on a part time basis through the school of Science and Health at Hawkesbury Campus at Western Sydney University. The overall aim of the research program was to examine cleanliness monitoring within healthcare settings, with a particular focus on the validation of rapid ATP testing. The experimental goal was to validate ATP testing as a reliable and quantitative mechanism for the assessment of surface cleanliness prior to any practical verification of the use of ATP testing within a healthcare setting.

This chapter sets out the background for the project, the development of the literature over the period of the project and the chronological development of the project. The stages of the project outlined include the validation of the rapid ATP testing systems, the development of an integrated framework for cleanliness monitoring arising from a risk assessment, and a final field based study within a healthcare setting which applied the teaching from the earlier project results.

Chapter 1 outlines the single and focused theme of the project across each of the five research publications to create a single body of novel work for this PhD project.

The Background to the project

At the time of commencement of the project in 2008, the problems of multi drug resistant organisms (MRO), and particularly methicillin resistant *Staphylococcus aureus* (MRSA) were recognised as a significant cause of morbidity and mortality through healthcare associated infections (HAI) in Australian hospitals.[1] There had been a dramatic increase in hospital cross-infections in many western countries over the past decade. In the United States, nosocomial infections of HAI were ranked among the top ten causes of death.[2] In Australia, it is estimated that 50% of all multiple resistant Staphylococcus aureus (MRSA) infections are HAI, with an 11% case fatality rate.[3] There is also extensive evidence of transmission of other bacterial pathogens such as Enterococcus species, including Multi Resistant Organisms (MRO) via healthcare settings, and particularly involving contaminated surfaces.[4,5]

Apart from morbidity and mortality, the economic impact is considerable. The annual number HAI cases in Australia is thought to be approximately 200,000.[6] A recent study estimated that a 1% reduction in HAI's should be attainable through a modest improvement in infection control and would free up 38,500 hospital beds annually.[7] The cost associated with HAI is a significant drain on the health care system and is estimated in terms of billions of dollars in the USA alone.[8] Equivalent cost data for Australia is not available, but the Australian Commission on Safety and Quality in Healthcare (ACSQH) acknowledges broadly that around half of all HAI are preventable through better infection prevention practices.[9]

The first tier response to the growing burden of HAI from the global clinical community, including Australia, was to focus on hand hygiene as a principle infection prevention intervention, and so the '5 moments of hand hygiene' campaign ('the 5 moments') was developed and accepted internationally.[10-12] 'The 5 moments' campaign recognised that bacteria can move via unwashed hands from surface to surface.[13] Studies had shown that where hand hygiene compliance was reduced the risk of nosocomial transmission of MRO increased.[14] The goal of 'the 5 moments' campaign was to increase the frequency of hand hygiene compliance amongst all health care workers (HCW) and particularly attending clinicians.

In Australia, hand hygiene products with bactericidal claims which are intended for hospital or healthcare usage, are controlled as medicines by the Australian Government regulator, the Therapeutic Goods Administration (TGA). The '5 moments' shifted the focus of hand hygiene onto the effective use of alcohol based hand rub (ABHR) products to speed up the efficiency around hand hygiene compliance and to allow bedside application of ABHR without need of a sink and running water.[12] The effective use of ABHR, with proven bactericidal properties, increased the likelihood that MRO that had been picked up on HCW hands via unhygienic surfaces, would be killed on the hands by the ABHR prior to transmission to another surface or object.

Whilst hand hygiene was the significant focus, no studies in Australia had considered the corollary issue of surface hygiene, and the effectiveness of cleaning and disinfecting of the patients' surrounds and the hygienic standards of cleanliness for general hospital surfaces. Whilst visual inspection was the primary cleaning and cleanliness monitoring method, it was suggested to define cleanliness standards in terms of bacterial contaminants present on healthcare surfaces.[15] Authors such as Dancer and Carling have noted that the microbes that are most frequently responsible for HAI are all recognised as organisms that can survive for long periods of time on environmental surfaces and that these surface are also the surfaces most frequently touched by healthcare workers.[16,17] More

recent work has demonstrated that these organisms can survive within dry surface biofilms on common hospital surfaces and can remain viable for many years.[18]

The former President of the Society of Healthcare Epidemiologist of America, Professor Robert Weinstein has commented that whilst a large amount is known about the genetics of organisms responsible for HAI, the precise mechanisms of development of antibiotic resistance and the interaction of these bacteria in the hospital environment continues to be poorly understood from a preventative perspective.[2] Environmental surfaces are recognised as an important reservoir for infectious agents of such nosocomial infections.[19]

One key study indicated that prior occupancy of the same bed or hospital room was a key indicator for the likelihood of a subsequent patient acquiring the same organism thus suggesting poor cleaning as a small but important causal link to HAI.[20] The issue of prior patient infection transmission risk was also more recently confirmed in a meta-analysis.[21] MRSA was identified as strongly associated with hospital surface contamination and nosocomial transmission when comparing environmental and patient isolates.[22] Gram negative bacteria are also responsible for HAI and are similarly associated with environmental contamination that could be controlled by improved cleaning processes.[5]

Some lamented modern hospital hygiene practices including disinfectant usage and harked back to the days of Florence Nightingale.[23] The need to reconsider hygiene standards and methods for the monitoring of cleanliness of healthcare surfaces required urgent attention.[16]

The TGA had instituted rigorous and mandated, pre-market, *in-vitro* testing requirements for hospital grade surface disinfectants since 1996.[24] Despite the regulatory controls, there was a view that disinfectants were both undesirable and unreliable, and additionally that cleaning was the key issue for effective hospital hygiene and cleanliness.[6]

The issue of disinfectant expertise within policy setting documents of the same period was a major concern. One authoritative Australian Government policy document contained poorly considered information on cleaning and disinfecting of instruments possibly exposed to prion contamination and this was criticised via a letter to the editor from this author (see Appendix A1).[25] A second document, published shortly thereafter by another Australian Government body, made recommendations on surface decontamination in regards to influenza virus which ignored the Australian Commonwealth legislation on disinfectants (see Appendix A2).[26]

Whilst disinfectants and their use were being ill-considered, it seemed that the important corollary issues around the hygienic status and the cleaning of surfaces (what gets touched and how to clean

them) were being uniformly overlooked and particularly so in Australia. The risk arising from this omission in approach was that disinfectant failure may have been solely as a result of the first chronological task of cleaning. No risk assessment was apparently conducted to identify this issue and even in relevant healthcare cleaning standards – such as the NSW Infection Control Policy of 2007 – the process of cleaning prior to disinfectant usage was not defined.[27]

In the 2010 Australian Infection Prevention Guidelines the cleaning process always precedes the disinfecting, and so to ensure that the disinfectants work correctly, it is important to understand the initial cleaning step as a quality assurance opportunity and eliminate cleaning as a variable for disinfectant performance.[6] To add to the confounders, whilst the disinfectant products are regulated by the TGA, the cleaning products are unregulated. The process of surface disinfection is therefore a two-step process involving an unregulated product use (for the cleaning process) followed by the use of a regulated disinfectant for the disinfecting process.

Cleaning of the environment has long been regarded as the essential first step in the major guidelines for Infection Control within health care settings and yet not one of these major documents defines the term “clean” in any quantitative manner.[6,28,29] The closest to a definition of “clean” is with the process of “cleaning” which is defined as “the removal of soil and a reduction in the number of micro-organisms from a surface”.[29] The primary shortfall with cleaning in hospitals in Australia and elsewhere is in the measurement of the efficiency and effectiveness of the cleaning processes due to the reliance on visual assessment alone for performance monitoring.

The primary mechanism for monitoring both what is ‘clean’ and also the process of cleaning is visual inspection. No other routine monitoring has been recommended in any of the major reference standards. Routine microbiological examination is not recommended in Australia or the USA.[6,28] If a surface therefore appears to be acceptably visually clean, and the audit tool developed in Victoria scores the surfaces as clean, then it is deemed to be clean.[27,30] There is no recognised or accepted, quantitative standard for what constitutes a clean surface, nor how it could be measured.[31]

The benefits of improved cleanliness or cleaning have been demonstrated. A study at Rush Medical Center in the USA with Vancomycin Resistant Enterococci (VRE) positive patients, demonstrated that with every 10% improvement in cleaning, there was a 6% reduction in the recovery of VRE within a contaminated ICU.[5] In a Scottish hospital with active MRSA patients and HAI – MRSA transmissions, the rate of transmission dropped significantly with the addition of just a single extra cleaning staff member.[32] Work with additional cleaning during an outbreak with a multi drug resistant Acinetobacter baumannii (MRAB) showed similar reductions in cross infections with cleaning enhancements.[33] The question is how to measure the cleaning and ensure that the standard of

cleaning processes regularly meets the standard of quantitative cleanliness that is desirable to reduce the risk of cross infections or HAI. The literature provided no clear answers on how to make cleaning improvements and permanently implement and provide a measure of quality assurance for these cleaning outcomes.

This gap in the literature around cleaning within healthcare focused the project onto the definition of healthcare surface cleanliness, and immediately two separate process issues became apparent. The first was the cleaning process itself, and the second was the outcome from the cleaning processes. Visual inspection and surface microbiology were insufficient to meet the requirements of a modern hospital. At that time, the literature suggested two new methods of cleaning validation for investigation. The two new methods proposed for applications within healthcare settings were the use of rapid ATP testing (ATP), and fluorescent marker technologies (FM), which is where the following description of the laboratory phase of the project commences.

Some Background on ATP testing

Adenosine Triphosphate (ATP) is present in all living cells and testing using ATP portable devices was first developed for the food industry.[34] The first published study using ATP testing to measure cleanliness within healthcare settings came later.[35] ATP is detected using the "firefly reaction" and the amount of light liberated in this reaction is roughly proportional to the amount of ATP present in a sample.[36]

The handheld and portable ATP testing devices measure all available ATP in a swab sample by firstly lysing any cells to liberate the ATP present, and then measure the light output from the luciferase reaction using a Photo Diode Array or a Photo Multiplier.[37] However, these devices do not distinguish between the sources of ATP and so any cells present (human, plant or bacterial cells) will yield a volume of ATP against which the light reaction will occur. Consequently, the use of ATP testing cannot be directly correlated with bacterial contamination, but only cellular contamination (all sources).

So, ATP testing is proposed a surrogate of cellular soiling in general, and not specifically bacterial soiling. To add to complications in use, ATP testing cannot distinguish on the basis of resistance or susceptibility to antibiotics (MRO). Review articles on the use and abuse of ATP testing have noted these shortcomings, which is discussed later and within the presented papers of this thesis.[38] The use of modern selective growth techniques and the application of modern genetics in microbiology (such as whole genome sequencing) are both essential for the detection and specific identification of

MRO as indicated later in the thesis. These improvements to specificity in microbial identification with MRO dramatically improve our understanding of the movement of MRO within and without the healthcare setting in both spatial and temporal aspects.[39]

The Initial Laboratory Phase

The use of rapid ATP testing had become available to the food microbiology sector in late 1998 with the innovation of hand held (portable) ATP testing devices and accompanying consumables. The use of rapid ATP testing was suggested in 2000 as a superior method to both visual inspection and microbial recovery methods for cleaning validation within healthcare settings.[35] This application of rapid ATP testing was being explored with field studies on the practical implementation of rapid ATP testing as a surrogate method for cleaning validation within hospital settings.[40-44]

The other new method of cleaning validation used a fluorescent marker (FM) and had been developed by Dr Philip Carling, an Infectious Diseases clinician who was concerned that critical cleaning required practical verification.[17] This new FM technology allowed tracking of cleaning action, and whilst being a qualitative measurement, the FM appeared to work well as a quality improvement technique.[45] The use of an FM was shown to aid in the reduction of *Staphylococcus aureus* nosocomial infection rates when included as part of a cleaning intervention during an outbreak of disease.[46]

The starting point for this PhD project at the Confirmation of Candidature (COC) was a research proposal which included the validation of cleanliness monitoring methods by comparing the new methods (ATP and FM) with the two existing methods (visual inspection and microbial recovery). The project would move from a laboratory based validation process (focused on rapid ATP testing and FM usage) and then move into a risk assessment that subsequently applied the findings of validation studies and risk assessment and tested these findings within a healthcare setting.

The initial studies arising from the COC project proposal quickly identified that the validation of the ATP testing systems required more laboratory based research than was initially anticipated. The first laboratory study subjected samples of the Fluorescent Marker (FM) to a range of drying studies to qualify the characteristics as described in the literature. A key observation was that the ATP devices were measurement tools (quantitative), whereas the FM was an observational (qualitative) tool.

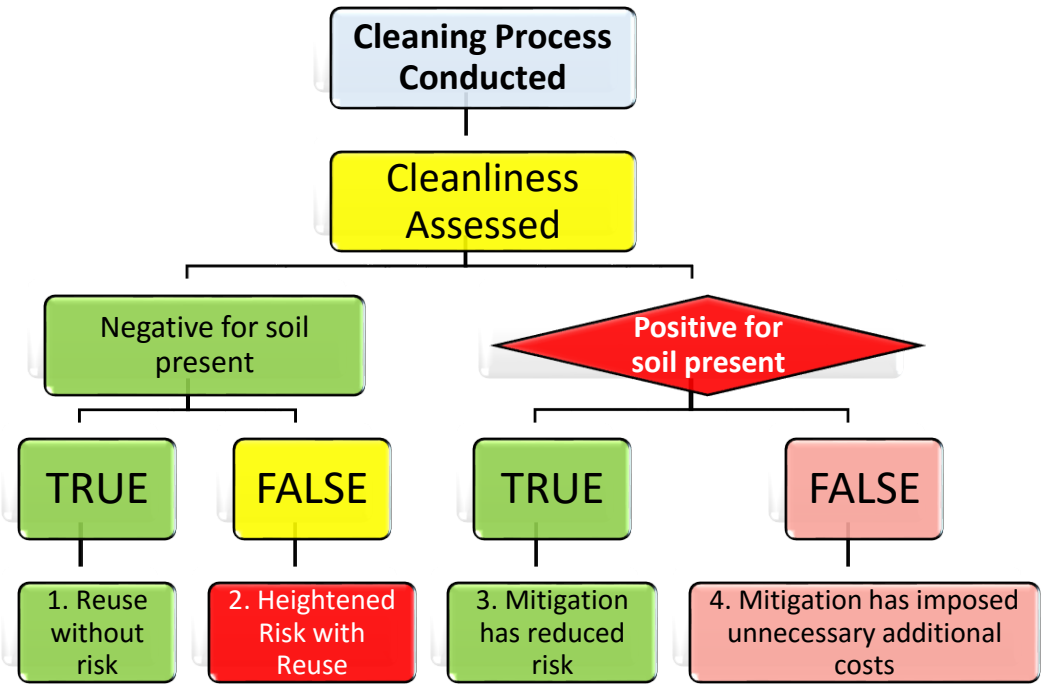
The initial experimental results were expressed through two Poster Presentations in 2011 at the International Federation of Infection Control meeting in Venice (Appendix B1 and B2). A result of the

early findings included a risk-based approach to cleaning monitoring which is shown on the poster titled “The importance of practical zero in cleaning performance indicators” (Appendix B1). The flow chart presented on the poster is shown as Figure 1.

The goal of a cleaning process is soil reduction or microbial removal and there are four possible outcomes from any monitoring process. The four possible outcomes are also individual risk elements of a cleaning process being true negative, false negative, true positive and false positive. Each of these risk elements has implications for cleaning management and infection prevention. The cleaning process involves a cleaning activity that should be measurable (efficiency monitoring) and the cleaning outcome that should also be measurable (efficacy monitoring). Whilst ATP testing measures the efficacy of the process, it is the role FM to measure cleaning efficiency.

The experimental results for FM were described on the second poster (“Validation of cleaning indicators for healthcare surfaces”) at the same IFIC 2011 Conference (Appendix B2).

Figure 1: Cleaning Process Risk Overview (*source: Whiteley Poster*: Appendix B1)



The Validation experiments with ATP testing devices and consumables

The laboratory work examining the validation of the ATP devices used a known source of standardised ATP in controlled dilution series. This experimental work used a new method to conduct the validation by applying the diluent directly onto the swabs using a micro-pipette. The study was based on standard validation methods as outlined in the International Conference on Harmonisation (ICH) Guidelines on validation for the pharmaceutical industry which calls for separate validation for each of the known variables.[47]

In using an ATP testing device, a pre-prepared swab (consumable) is used to wipe across the surface to be tested, which is then recapped, and the reagent (which is contained in the proximal end of the swab) is released onto the swab which allows the ATP-Luciferase reaction to initiate. There is a short activation time of around 15 – 30 seconds during which time the swab is inserted into the ATP measurement device for detection of the light arising from the reaction. The light is measured by the ATP testing device and the measurement is expressed as relative light units (RLU).

The first and primary consideration in the validation process for the rapid ATP testing devices and their consumables is the reliability of the core chemical reaction between the Luciferase and the ATP and the quantification of RLU expressed as a result of the reaction. The initial validation started with testing the dose-response of the devices using this core chemical reaction process before any other variables were introduced to the validation experiments.

The additional variables present when using a rapid ATP device and consumables as intended, include the swab 'material', which was identified as an independent variable in its own right (the first independent variable in addition to the core biochemical reaction). The swabbing process is a separate, second independent variable. The reagent containing the luciferase must also contain a separate chemical reagent which will induce cellular lysis to liberate the intercellular ATP, and this lysis process is yet another and third independent variable. So, before the essential reaction can occur, there are at least three separate variables which could each independently effect the results of the validation.

In order to avoid confronting these variables, the decision was taken to use a calibrated micro-pipette which would apply the ATP in quantitative volumes directly onto the swabs, thus focusing the testing purely on the performance of the devices/consumables against pure ATP solution in a quantitated dilution series. This was a previously unpublished method for this type of validation study and has subsequently had citations in peer reviewed publications looking into ATP studies.

The results of the study were therefore a more accurate indication of validation of ATP devices than any prior studies, all of which were either field based or subject to the combination of all four variables (dose response; swab material; swab technique; and lysis chemistry). The other key issue arising from the research was that a sufficient number of replicates at each dilution point were required to establish precision at each sample point which was also a previously unreported finding. This was an important observation due to the variability observed in the first experimental phase.

The results from the first validation study of the three ATP bioluminometers were analysed and submitted for publication. The first full paper arising from the laboratory studies on the three ATP devices was submitted to the journal, Healthcare Infection (CSIRO publications), was accepted into peer review in June 2012 and was accepted for publication in July 2012.[48]

The key findings of the first paper were threefold. Firstly, the scale used to express the measurement of ATP uses Relative Light Units. Whilst all of the commercial ATP devices make use of the same equation (one ATP unit plus one Luciferase Unit gives one light unit), each of the devices applies a different algorithm to express the measurement of light units and even the method of light detection varies between different branded ATP devices.[37] So, whilst the same equation is used as the underlying technology, the expression of RLU is relative in every sense. There is no standardised scale and so each ATP device will express a quite different number when measuring a standardised ATP quantity. The lack of a standardised scale means that different brands currently have no interoperability. The lack of a standardised scale also means that any calibration tool is relative to that device only and has no universal applicability. Without a standardised measurement scale, accuracy of measurements cannot be assessed.

Secondly, the ATP devices were reasonably reliable when testing against a ten-fold dilution series (and taking the median reading as indicative). An important contribution of the findings arising from the log scale dilutions was that all three ATP devices tested did demonstrate a uniform linearity of response over the core part of the dynamic range. However, all of the ATP devices tested were highly unreliable at testing a 20% dilution series. The uncontrolled variability revealed through a standardised laboratory validation process was both surprising and unexpected. Interestingly the use of medians to express a group of readings was more reliable than averages due to the frequency of outliers (which was also part of the novel result).

Thirdly, there was no way for a normal user to detect the level of variability when using an ATP device. Therefore, the applicability of ATP devices to perform a role in validation of cleaning was undermined by the limitations of the devices.

Lord Kelvin once wrote:

"*I often say that when you can measure what you are speaking about, and express it in numbers, you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind; it may be the beginning of knowledge, but you have scarcely in your thoughts advanced to the state of Science, whatever the matter may be*." [49]

The basis of measurement sciences is that the numbers produced can be relied upon to accurately express the underlying measurement. The first paper arising from this study demonstrated that this fundamental principle of reliability of measurement was seriously compromised when using rapid ATP devices. Further work was required to understand and quantify the level of variability.

The work on reliability was extended in the second paper by using a simple but elegant statistical tool known as the Coefficient of Variance (CoV or Cv).[50] Cv divides the standard deviation into the mean and gives a number that should be always between zero and one. The lower the number, the lower the variance. The Cv is the inverse of the engineering equation most frequently known as the signal to noise ratio (mean divided into standard deviation).

Fortunately, the first experimental series used a large number of replicate readings at each concentration point, for each of the ATP devices. From this data set, the mean and standard deviation were calculated for each discreet concentration point with that associated sample group (same ATP device, same concentration and multiple readings at that concentration) and from there the Cv was calculated for each ATP device at each concentration.

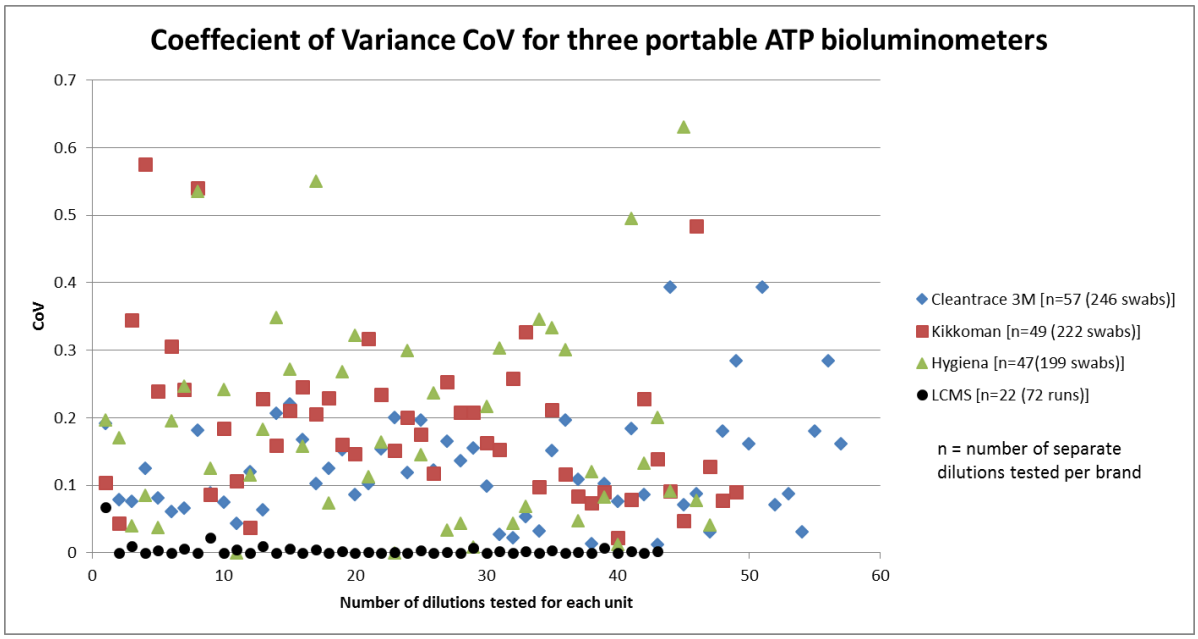
In this first experimental series a standardised and validated laboratory analytical tool (HPLC option on the Liquid Chromatograph – Mass Spectrometer, or LCMS) was also used to measure standardised ATP, and this provided a comparative data set. The Cv for the HPLC was very low, whilst the Cv for each of the commercial ATP devices was routinely high.

Another advantage of the Cv over the more frequently used standard deviation, is that whilst the standard deviation is unique for each of the discreet sample groups, the merging of Cv and average into a single ratio, normalised all of the data from each of the discreet sample groups, thus allowing a true comparison. Figure 1 from the second paper is shown below in colour to illustrate the problems of variability observed in this first experimental series.

The authors' initial experimental studies on the ATP testing devices demonstrated that instrument performance required additional consideration in regards to validation and to practical use. A limitation of the first laboratory study on ATP testing devices which provided the data for the first and second published papers, was that the testing was limited to only pure ATP solutions. This

opened a reasonable opportunity for additional study using bacteria. Studies were commenced using bacteria to challenge the performance of ATP testing devices.

Figure 2: Coefficient of Variance for three portable ATP bioluminometers (*source: second Whiteley Paper*: ICHE 2013:34:538-540)



Consideration of Failure Risk arising from Cleaning Monitoring Methods

Whilst this laboratory based research into ATP device variability was continuing, an important strategic question arose in regard to the findings. The initial two publications had demonstrated that the reliability of measurements was an underlying and uncontrolled variable for the field use of ATP testing within healthcare settings. The next question was to consider the impact of the published findings (from the first two papers) on current uses of the ATP testing devices within healthcare settings. The impact of this unreliability in measurement required a risk assessment.

The earlier work outlined in one of the authors 2010 posters ("The importance of practical zero in cleaning performance indicators") mapped out a risk management approach that accounted for both false positive and false negative interpretation risks from the data (Appendix B1). These could be considered as type I and type II statistical errors. This methodological approach had been used in studies attempting to deduce a 'best option' for a cleanliness threshold using ATP testing where a

Receiver Operating Curve (ROC) was developed to position the proposed cleaning threshold based on a proposed RLU level.[51]

A risk-based study was undertaken to consider the impact of the variability of the ATP testing devices in practical use within healthcare settings. Given that failure risk can include both false positive and false negative outcomes, and that failure risk applies to all of the cleaning monitoring methods, the study was widened to also include FM, visual inspection and microbiological recovery methods.

The use of each of the monitoring methods was considered using the innovative application of Failure Mode and Effects Analysis (FMEA), which is a standard tool used in the medical device manufacturing sector.[52,53] Whilst this focused the study onto the failure risks of each of the methods, the literature revealed that these risks were more clearly identified than any positive association with any of the cleaning monitoring methods.

Far from being a negative study (as implied by the word 'failure'), this FMEA process allowed the combination of the different methods to reveal a new approach and integrated use of each of the cleaning monitoring methods (see Figure 3 below).

This integrated monitoring approach reflected the risk overview shown in Figure 1 from the first poster in 2011. In that earlier figure, the four possible outcomes from any cleaning process were outlined as true positive, false positive, true negative and false negative (indicated as the worst possible failure risk in red colouring). In this third paper, the monitoring methods were combined into an integrated framework so as to minimise the risks of any one method providing a false negative outcome (this would be an indication of cleanliness where in fact pathogenic microbes remained despite the cleaning processes).

The third paper, which used the FMEA approach was submitted for publication in August 2014 and accepted for publication in October 2014.[53]

This FMEA study linked all of the monitoring methods, including the ATP testing, and contextualised the application of the cleaning monitoring methods in a new and novel way. This work both built on the ATP validation work and extended a justification for continued use of ATP testing despite the risks of measurement variability. The work also demonstrated that ATP testing has a distinct role in cleaning monitoring that is unique in its timeliness, quantitation of cleanliness, and ease of use.

Figure 3: An integrated model for cleaning monitoring (*source: third Whiteley paper*: AJIC: 2015:43:147)

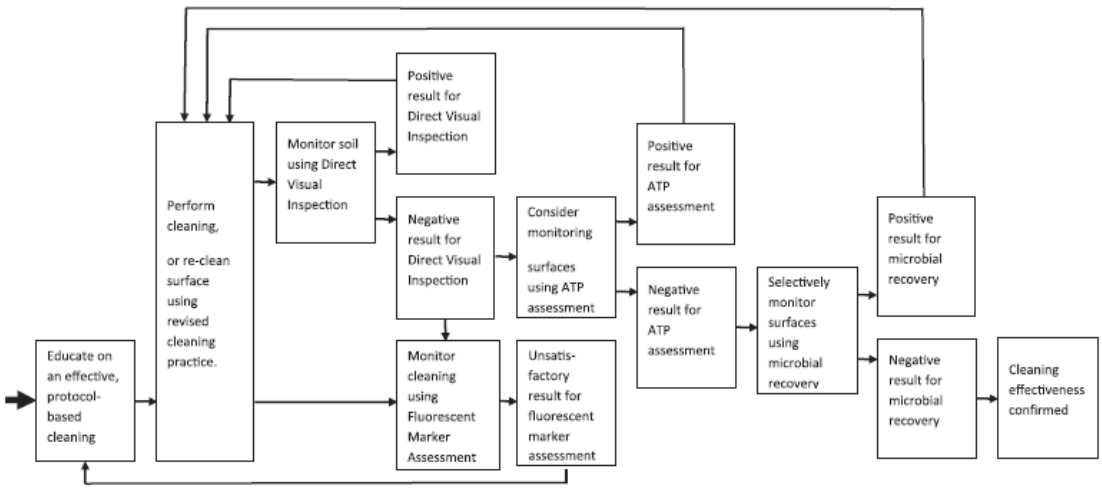


Fig 1. Model for cleaning monitoring.

Continuing the Laboratory Phase

Experimental work on the validation of the ATP devices was extended to also consider the detection and measurement of bacterial ATP. Given that the swab materials and lysis chemistry were fixed (each determined by each of the ATP device manufacturers) and that the swabbing techniques required for environmental cleaning were well reviewed by others, the focus of the remaining laboratory work with ATP devices was on the essential responsiveness of the ATP devices to the suspensions of bacterial cultures. This extended the experimental phase using several known bacterial species in quantitated dilution series. These experiments with suspension cultures were used to examine the variability of the devices ATP-Luciferase reaction and to compare this to the variability observed when only pure ATP had been tested.

A number of experimental runs using different bacterial species were commenced. Bacterial cultures were selected on the basis of gram staining and general relevance to healthcare settings. The bacteria initially chosen were laboratory strains of *Staphylococcus epidermidis* (ATCC 12228) (*S epidermidis*), *Escherichia coli* (ATCC 25922) (*E coli*) and *Pseudomonas aeruginosa* (ATCC 15692) (*P aeruginosa*).

The use of bacterial species required an extensive period of range finding and experimental exploration in order to develop sound techniques and approaches to underpin the microbiological

validity of the project. Refreshing the benchtop microbiology methods was an important personal feature of the journey. The preparation period was extensive and ensured that repeatability in the results was attained, including issues such as quantitation of the dilutions of the suspension cultures.

The microbiological work added materially to the validation process as range finding work confirmed the new variables to be controlled. An example of the value of the preliminary work was that the use of *E. coli* was discontinued following what appeared to be lysis failure for both of the 3M and Hygiena ATP devices/consumables. Another validation issue was that the use of Triptone Soy Broth (TSB) as a growth media for the suspension cultures was observed to interfere with two of the devices/consumables (Kikkoman and 3M) whereas Phosphate Buffer Solution (PBS) had no impact. The use of TSB was thereafter discontinued in preparation of the bacterial dilution series for validation testing.

An additional (fourth) commercially branded ATP device was made available through the University of Tulsa in Oklahoma and the Cleaning Industry Research Institute International in the USA. This ATP device (Charm Inc) added a new dimension to the work as its scaling was much higher than the existing three commercial devices used in the earlier studies.

The methodological approach for the microbiological challenge to the ATP testing devices followed the earlier work using pure solutions of ATP as used in the first study. The statistical analysis using the Cv approach used in the second study was also followed and so the results of this extended laboratory phase of the validation work were all translated from the raw data into Cv results.

The initial studies using pure ATP dilutions were also repeated to ensure comparability and completeness, although data from all of the pure ATP dilution series of studies was included in the full presentation of Cv analysis. Statistical advice was obtained through Mr Paul Fahey who also agreed to be included as co-author on the paper.

The results from the studies using bacterial cultures demonstrated that the variability observed using ATP from a known source, was precisely the same variability levels seen when testing against bacterial species. Whilst the lysis variable was still uncontrolled, the results mirrored the earlier finding on variability.

The fourth Whiteley paper was written and then in November 2014 it was submitted to the leading healthcare epidemiology journal, Infection Control and Hospital Epidemiology (ICHE) which is published on behalf of the Society of Healthcare Epidemiologists of America (SHEA).[54] This paper confirmed the experimental findings on variability and demonstrated the importance of a thorough validation approach prior to any field based applications of ATP testing and interpretation of the data arising. This study is the first published paper to demonstrate that the variability seen when

challenging ATP testing devices with pure ATP solutions was exactly mirrored by the variability observed when testing those same devices against quantitated bacterial dilution series'.

Communicating the message on ATP testing variability

During the period of writing up this fourth paper, a study by Visrodia et al., was published (also in ICHE) which used ATP testing to 'validate' endoscope cleaning.[55] The work was supported with funding by the commercial manufacturer whose ATP testing device was exclusively used in the study. The paper failed to provide validation data and used a single point sampling method, which overlooked potential variability, flaws in the sampling methodology and statistical analysis.

A response was initiated by this author and a letter to the editor was submitted for publication in September of 2014 (accepted October 2014), which focused on two problems with the Visrodia paper.[56]

Firstly, the issue of using a single brand and not cross correlating with a second branded unit was discussed to illustrate the dangers of the relative scaling and lack of unit interoperability. A commentary on the Visrodia paper had made a similar, albeit unreferenced remark.[57]

Secondly, the Visrodia paper indicated that the sampling method used to obtain ATP readings was based on single point samples (not even duplicate sampling was conducted) and therefore variability issues were unrecognised. The statistical analysis of the raw data was done on this sample set and found with significant findings. This raised the significant risk of type I statistical error.

In the reply from Visrodia et al., the most important responsive comment was "*Given the imperative for cost containment and to improve efficiencies on the front lines, we believe it would not be desirable to perform duplicate or triplicate testing as suggested by Whiteley et al. Their concern about variability within and between ATP measuring devices deserves additional study*." [58]

The fourth Whiteley paper, which focused specifically on the issue of recurrent variability in ATP testing, was published just three ICHE editions later in the June 2015.[53] This fourth paper set out the critical issue of variability and for the first time proposed a quantitated risk of misreading based on the high variability. Earlier mathematical work by Reed et al., had proposed that where the Cv is above 0.4, then there is a 20% likelihood that any one reading (in RLU) could be out by a factor of two.[59] In this context a reading of say 100 RLU, could indicate a true reading of between 50 RLU or up to a true reading of 200 RLU. This gave insight into the variability observed in the first Whiteley paper when the 20% dilution series were undertaken.[50]

In that first study, the log scale differences had minimised the impact of this variability (difference is '10-fold' and not just '2-fold').[50] Whereas by using a 20% dilution series the RLU readings presented in figures 2 – 4 in the first published paper, showed that there was often overlap between what should have been clearly differentiated readings/data sets.

This insight also goes some way to explaining why many of the statistical findings on earlier studies by other groups produced insignificant results, and results which did not correlate well with parallel measurements such as microbial recovery. Whilst the ATP testing does read a wider variety of ATP soils, and not just bacterial ATP, the results often obtained were incongruous.

A good example is in the paper by Sherlock where in several instances the ATP readings go up after cleaning whilst the microbial results decrease.[60] In Lewis et al., readings using an ATP cleanliness threshold of 250 RLU (a 3M ATP device) also often show failures where below threshold microbial growth is noted.[61]

To further add to the confusion and variability difficulties with interpretation of results from ATP testing, there is no standardised size of sampling area for swabbing. When ATP testing was first suggested by Griffiths et al., the sampling area suggested was a 10x10cm ($100cm^2$) area with a target level of cleanliness of 500 RLU using a Cleantrace (3M) ATP testing device.[35] Following studies using the same branded equipment and same sampling area concluded that the cleanliness standard should be reassessed and suggested a cut-off of 250 RLU.[61]

A study by Andersen et al., suggested that the cleanliness standard be reduced to just 100 RLU using a similar 10x10cm = 100 cm2 sampling area but using a different brand of ATP testing equipment (Hygiena).[62] The swabbing area for use in ATP testing has often followed the recommendations of a $100cm^2$ area (10cm x 10 cm) in both food and healthcare settings whilst using non-comparable equipment. [63,35,43,62] Another study selected a smaller sampling area of just $16cm^2$ (e.g. 4cm x 4cm = $16cm^2$).[64] Finally, another study using the Hygiena ATP reaffirmed the 100 RLU cleanliness threshold, but reduced the sampling area to a more practical 2 x 5 cm ($10cm^2$) area.[51]

The variability problems with these useful little devices 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.

Another approach taken in an effort to determine a reliable RLU 'cut-off' is the use of an ROC curve correlated with bacterial recovery from similar surfaces. The work in Mulvey et al., used the ROC curve approach and concluded that this method provided only 'weak support' for the 100 RLU cut-off.[51] Similarly, in Smith et al., after application of the ROC curve approach, using a different ATP testing device (Cleantrace, 3M), the ROC supported an elevated RLU threshold or cutoff at 800 RLU

on a 10x10cm=100cm^2 sample area.[65] This threshold outcome was not able to be correlated between the three cleanliness monitoring methods used in their study.

The issues of different RLU scales, unpredictable readings in RLU readings due to inherent variability, the non-standardised swabbing area used in differing studies, the poor correlation with bacterial presence on surfaces, the weak evidence provided through methods such as the ROC curve approach, and the differing performance characteristics between ATP testing device brands can all work together to confound practical studies using ATP testing. The practical reality is that a pre-determined, single cleanliness threshold expressed in RLU, which is common to all ATP testing devices is statistically unobtainable at this point in time. Any field based study requires very careful consideration to mitigate these issues, which was the point made in response to Visrodia et al. [56]

The practical application of the findings through a (pilot) field based study

In August and September 2014 an opportunity arose to use the ATP testing devices in a practical field application in a real healthcare setting. This study integrated the earlier findings with an existing clinical microbiological research program through the School of Medicine at Western Sydney University. This study – lead by Professor Iain Gosbell – was investigating the locations of MRO embedded within dry surface biofilms which are present within an Intensive Care Unit (ICU).[66, 18]

The first sampling attempt to locate the biofilms used a list of likely sites and objects within the ICU. This sampling round used only microbial recovery and required a more aggressive form of surface swabbing based on a method originally proposed in ICHE by Corbella et al., whilst that Corbella team were searching for *Acinetobacter baumannii*.[67] The biofilm research was interested in locating any multi drug resistant organisms (MRO) imbedded within biofilms on environmental surfaces. To recover the MRO a selective growth media was used, followed by further identification methods.

However, the initial results from the first sampling round were poor with only a single sample from twenty three total samples returning positive growth on the selective media.

The candidate posed the question: 'Could the ATP testing assist in the identification of more dirty areas when used in combination with the microbial recovery methods as per the teaching of the third Whiteley paper?' [52] The sampling methodology in this pilot study used both ATP and surface microbiology at matched sites within the ICU. The sampling method used two of the ATP testing devices at each sample location and in tandem with the aggressive biofilm swabbing approach.

In the second sampling session – a trial run – the sampling approach using two ATP devices and microbial recovery. Whilst at every sampling site one of the sampling methods was used (either of

the ATP devices and sometimes microbial recovery), at only a limited number of the sites were all of the three methods used. The two ATP devices used were the Hygiena and Kikkoman devices and accompanying consumables. The Hygiena device was used more extensively in this initial ATP sampling session which followed on from teaching by Andersen et al.[68]

A third sampling run was organised for early in September where all of the samples taken were fully matched. The collaborative approach provided excellent results.

The statistical analysis indicated that the ATP testing did assist in identifying the surfaces with higher levels of microbial soil (Chi-Square P>0.025[corrected for Yates] and Fishers Exact P=0.01). The sampling sequence was 1/23 MRO positive samples on the first round, with 12/36 MRO positive samples on the combined second and third sampling rounds when the samples were fully matched.

Importantly, the sampling locations with higher ATP readings in many instances grew MRO, although on the day of ATP testing and microbial sampling, it was only the ATP testing in real time which gave the indication of the more soiled surfaces with higher RLU readings. The microbial recovery results were not known for five days after the sampling, and longer for the accurate microbial identification of the specific MRO which were recovered.

Whilst the more aggressive sampling method did assist in higher recovery rates than normal swabbing methods might have achieved, the statistically significant findings demonstrated that using a list and visual observation, was poorly successful (1/23). Whereas, with real time feedback via an ATP testing device (where the matched samples were conducted), sampling revealed a large number and variety of MRO on environmental surfaces available to potentially infect patients (12/36). This use of a combination of the recognised cleaning monitoring methods largely followed the teaching of the earlier work in the third Whiteley paper.[52]

The results in real time of the ATP testing, indicated that the focus of the sampling should be moved from the patient surrounds to the area around the clinical work station including chairs and highly touched devices with which staff were in frequent hand contact. This proved a decisive approach despite concerns over the use of ATP in healthcare settings.[69] The discussion section of the published paper identified a number of potential flaws in interpretation of the results but also indicated improvements for further and on-going studies to improve infection prevention strategies focused on environmental surface hygiene within healthcare settings.

The fifth paper was submitted to the American Journal of Infection Control for publication in June 2015 and was accepted in July 2015. It was published in the December 2015 edition of the journal.[70]

This fifth paper is the first Australian study to have located such a high number and variety of contaminated surfaces (13 separate locations) with such a variety of MRO inside an active ICU.

The paper was written with a clear outline of the flaws in the pilot study, but with the statistical findings clearly described and discussed.

The paper also made some other very important observations. Using two ATP devices in parallel within a healthcare setting has been documented in only one other paper.[68] The paper by Andersen et al., used a 3M device and a Hygiena device. Whilst the Hygiena device provided a platform of results that were significant for some measurements, the 'before-and-after-cleaning' readings from the 3M device were not statistically significant. Unfortunately, there was no attempt in the Andersen paper to correlate the ATP readings (in RLU) between the two ATP testing devices.

In this fifth Whiteley paper the correlation between the two devices was subject to direct comparison. The first important finding of this paper in respect to rapid ATP testing devices and their variability was the almost zero correlation (r^2=0.0144) between the sampling results of the two branded ATP devices – Hygiena and Kikkoman – that were used in the study. Whilst the manufacturer of one of the ATP devices used (Kikkoman) does note the capacity of the consumables to measure other components (including Adenosine Mono-Phosphatase = AMP) this finding warrants additional research in subsequent studies.[71]

The second important observation made in the fifth paper relating to ATP testing, was that provided that the teaching of the third Whiteley paper (using FMEA) was followed with parallel/alternative cleanliness measurements, rapid ATP testing did contribute significantly towards indicating surface cleanliness. This is a very positive finding for the use of ATP testing when examining the cleanliness of surfaces. The use with biofilm studies also warrants additional and future research.

This fifth paper is a neat conclusion to the work. As a pilot study the paper demonstrated the learning achieved through this confluent body of work. It takes the findings of the earlier validation and risk studies and extends them practically in a new and novel manner with outstanding results, all based on the findings of the four other (included) Whiteley papers.

The essential goal of cleaning monitoring is to ensure that the cleaning is achieved both in terms of efficacy and efficiency. FM can play a qualitative role in efficiency monitoring as indicated in the posters presented from the earlier project findings. The use of rapid ATP testing as presented in this fifth paper provides a timely (rapid) indication of surface cleanliness that allows further intervention or investigation if required. The validation work achieved through this study has demonstrated both the problems and failure risks with commercial rapid ATP testing devices (as they are currently

configured). The implications arising from this observation contrast earlier critical for the use of rapid ATP testing within healthcare settings.[72]

The validation work also contextualised these flaws when ATP testing is used within the healthcare setting, and outlined how the flaws can be minimised, whilst taking full advantage of the benefits of the ATP testing and real time readings.[73] The final and fifth paper demonstrated how the variability was both present in the study, and yet the real time impact and cleanliness indication was sufficiently useful to direct microbial sampling to uncover a wide array of MRO lurking in plain sight and within the clinical area of an active ICU.

The following chapters set out:

a. In Chapter two, the overview of the methods used in this thematic study;
b. In Chapter three, how the five papers demonstrate the work and meld together into a single PhD thesis as a complete body of novel findings; and
c. In Chapter four, how the work has opened up a wide new pathway of further and on-going research.

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Chapter 2

Methods used within the study

Précis

This chapter outlines the background for the methods used within each of the five papers and explains how these methods interlock in a single confluent theme within the PhD. The full details of each of the methods are described in each of the five papers. This chapter sets out the salient reasoning for selection and development of each of the methods and the contextualisation for each application. This chapter also discusses the implications of the methods for further investigation into the use of ATP testing for cleanliness monitoring within healthcare settings.

A brief outline of the methods used in the five papers

The backbone of the study is the laboratory phase which investigated the validation issues for rapid ATP testing. The laboratory methods followed the applicable principles of pharmaceutical industry guidelines to establish the responsiveness of the ATP testing devices and their consumables.[1] The data were generated through the novel 'direct droplet' method of analysis whereby ATP was directly applied to each of the ATP swabs using a micro-titre pipette. The data were then plotted, statistically analysed for association and also analysed the using coefficient of variance (Cv) method. The use of this approach provided a basis to normalise the data across the full array of the experimental series and to compare each of the branded ATP devices.

The first, second and fourth of the papers included in the PhD study outline the methods of analysis for the ATP responsiveness and the results obtained from the testing program. The ability of the ATP devices to meet the core validation criteria of linearity, dynamic range, accuracy, precision and limits of detection are demonstrated in the results. The methods used demonstrate that uncontrolled variability is an issue for all of the ATP device brands tested in this study. A key quality assurance concern for any application of ATP testing within healthcare settings, is that the measurements provided by the ATP testing must be reliable and well understood so that interpretation of the ATP data is able to stimulate an appropriate quality assurance response.

The second phase of the work, as outlined in the third paper, took the data from the ATP testing and considered how to integrate ATP testing into a cleanliness monitoring process. The study used the risk based method of 'Failure Mode Effects Analysis' (FMEA) to assess the impact of the hazards of cross infection where any of the four main methods of cleanliness monitoring are used. A novel

proposal for an integrated cleanliness monitoring approach within healthcare settings was a key finding arising directly as a result of the novel application of this FMEA method.

The fifth paper outlines the final phase of the work where a field based pilot study was conducted. This study followed and applied the findings arising from firstly the ATP validation studies and secondly the new integrated cleanliness monitoring process. This pilot study was conducted within a working Intensive Care Unit (ICU) in a modern teaching hospital. The sampling method used two ATP devices, a novel approach to microbial sampling, and the statistical analysis compared the findings when using microbial sampling only, with the integrated use of visual inspection/observation, microbial sampling and rapid ATP testing.

Table 1: The methods used in the Five Whiteley papers

Paper Number	Abbreviated Title	Journal, Year	Method of Analysis	Statistical Analysis
1st Paper	Comparative performance of 3x ATP Bioluminometers	HI, 2012	1. ATP analysis using ATP devices - using pure sourced ATP; 2. ATP analysis using HPLC	1. Test of association (Kruskal-wallis) 2. Linear correlation (HPLC) 3. Box and whisker graphs for precision testing
2nd Paper	ATP Device Reliability Testing	ICHE, 2013	Used data from 1st paper	Coefficient of Variance (CoV or Cv)
3rd Paper	FMEA & Synergies	AJIC, 2015	Risk analysis using FMEA	Not applicable
4th Paper	The Perennial problem of variability of using 4x ATP bioluminometers	ICHE, 2015	1. ATP analysis using ATP devices - using pure sourced ATP; 2. ATP analysis using 4x ATP devices using 2x bacterial suspension cultures; 3. ATP analysis using HPLC	1. Coefficient of Variance (Cv) 2. Box and whisker graphs to indicate data spread
5th Paper	Pilot study in finding the bad bugs in an ICU using ATP & Microbiology	AJIC, 2015	1. ATP analysis using 2x ATP devices in an active ICU setting 2. Microbial recovery using swabbing and selective media with ID specificity analysis (Vitek)	1. Test of association using Chi-square and Fishers Exact Test; 2. ROC Curve (sensitivity analysis); 3. Linear correlation with results with the 2x ATP devices

Key: Abbreviations: HPLC = High Powered Liquid Chromatography; ICU = Intensive Care Unit; ROC = Receiver Operating Curve
Journals: HI = Healthcare Infection; ICHE = Infection Control Hospital Epidemiology; AJIC = American Journal of Infection Control

In each paper the methods of analysis and statistical methods were overseen and re-checked with supervisors and co-authors for the papers. In the first 3 papers the primary author was Whiteley plus two co-authors (Dr Derry and Dr Glasbey). In the 4th paper a third co-author was added (Paul Fahey). The 5th and final paper had the principle author (Whiteley) and one of the prior co-authors (Dr Derry), plus four additional co-authors (Jessica Knight, Professor Gosbell, Associate Professor Jensen, and Associate Professor Vickery).

The methods of analysis and statistical and drawing tools used in each of the five papers are shown in Table 1 where they are briefly outlined.

Methods of analysis for ATP testing devices (Whiteley Papers 1, 2 and 4)

The method that underpins the first three of the papers used a novel approach to measuring the accuracy of the dose-response for the application of ATP or ATP containing soils with the various ATP testing devices and their associated consumables. This new approach used a calibrated pipette to directly and quantitatively apply the test solutions directly onto each of the ATP device swabs. Until the first of the papers in this PhD, all of the studies in healthcare had used bacterial suspensions, either applied to a surface or dried onto surface, to assess responsiveness and other characteristics of the rapid ATP testing devices.

In the period leading up to the initial study (the first paper), only one paper had been published in infection control journals which used a validation style of approach to ATP testing devices.[2] In this paper by Aiken et al, the test solutions were bacteria dried onto a surface.

The Aiken paper used only a single microorganism (*Staphylococcus aureus*) but tested a range of ATP bioluminescence tests (including rapid ATP devices) and the results indicated many inconsistencies in the ATP results including a large variance in results and a reported Cv of '133'. The results compared readings in RLU and ATP light emissions across a dilution series on a log scale (ten-fold dilutions) and concluded that ATP bioluminescence was not an accurate way to measure the number of bacteria on a test surface. The work also noted that the number of bacteria was frequently underestimated (by a factor of up to 10 times) and that the highest level of inaccuracy was at the lower levels of bacteria being present.

In early 2012 a paper by Shama and Malik reviewed and strongly criticised the use of ATP testing for hygiene monitoring.[3] The authors engaged in a series of letters which responded to a series of papers on ATP which were published without suitable reference to the difficulties of using ATP testing and particularly in response to articles propositioning the ATP testing and use of cut-offs of

various RLU values.[4] A paper by Heller et al., also presented data which suggested that the 250 RLU cut-off did not correlate well with the presence of MRO on hospital surfaces using a Neogen ATP testing device.[5]

Another of the papers in 2012 examined the comparative reading and reliability efficiencies of three branded ATP devices using three microorganisms across a log scale dilution series.[6] This work by Sciortino et al also used microorganisms dried onto surfaces. This paper also identified that consistency was a problem for all brands, although a 'best brand' was selected. A close examination of the results suggests that variability was a constant problem and that variables such as swab material, swabbing efficiency and even lysis efficiency were uncontrolled and unrecognised until later in the experimental phase.

The authors also hinted that use of a direct droplet application onto the swabs may produce a different result from application of the swabs to a dried inoculum on a surface, but there were many 'random effects' noted in the paper. The reasoning for this conclusion was based on the response of one organism (*Candida albicans*) which showed higher readings using dried inoculum rather than directly applied volumes of a suspension culture. As a retrospective reading of their data, the authors suggested that swabbing in combination with the lysis agent may in itself liberate bacterial ATP, however this was unproven, and somewhat questionable given the other inconsistencies in the various results. The important findings from the paper were that the three ATP bioluminometers all read on different RLU scales, and that the sensitivity of response to different bacteria was somewhat brand specific.

There was an interesting exchange between Dr Malik and Dr Sciortino in a subsequent edition of the journal in regards to the validity of the results presented in the paper.[7, 8] Dr Malik warns against overstating the case in favour of ATP testing and cautions explicitly against using ATP testing as "…a surrogate indicator of microbial burden and hence risk of infectious agents".[7] The response from Dr Sciortino defended their laboratory validation, but threw the issue of protocol development and validation for field use back onto the hospital staff thus leaving unresolved the practical issues of implementation for ATP testing within healthcare settings.[8]

This highlighted the difficulties in the use of ATP testing for practical cleanliness monitoring purposes. Whilst the ATP devices are broadly and proportionately reactive to bacterial ATP in any series of ten-fold concentration dilutions, the practical applications require reliability on comparative cleanliness including detection of bacterial contamination.[2,6,3-5] To move the ATP testing from the laboratory validation into use for cleanliness monitoring, reliability was the significant concern as any over-reaction in ATP readings could have cost implications (additional cleaning

required), whilst any under-reaction might allow infectious organisms to persist despite cleaning processes. Reliability in the precision of response of ATP testing devices to ATP was identified as an issue for exploration.

In the work for the first Whiteley paper, the first research goal was to focus on the reliability of the ATP reaction response by the ATP devices to be tested. To achieve this outcome it was necessary to remove or minimise (as best as possible), all of the extraneous variables, including swab material dynamics, swabbing efficiency, lysis efficiency and even species sensitivity. It was agreed that the use of a pure (known and validated source of ATP) would allow the examination of just the 'ATP + Luciferase' reaction. A ten-fold dilution series of pure ATP which was directly applied onto the swab via a calibrated micro-titre pipette, removed the non-essential variables and focused the results on only the reaction dynamics.

As an extension of this work and to test the precision of response (by the ATP devices), on a narrower basis, a 20% dilution series was also tested. This provided additional insight into the precision around smaller variances in ATP concentration.

The use of a fully validated HPLC method, anchored the findings in terms of a "gold standard" against which the precision of the ATP devices could be compared.

This novel direct application method was used as the basis for all of the ATP data in the first, second and fourth Whiteley papers. This included using the direct droplet method for the experiments using suspension cultures of bacteria.

For each experiment, the suspension culture was diluted from a freshly grown laboratory stock of the selected bacteria. Confirmation of live bacterial was performed using serial ten-fold dilution onto agar plates. Each of the experiments were repeated on multiple separate occasions to ensure repeatability. The data arising from the final set of repeats using bacterial suspensions were used in the 4th Whiteley paper. The work using pure ATP and the four ATP testing devices was also repeated to ensure that all of the ATP devices available for the validation work were considered as part of the final validation paper (the Charm ATP device had not been used in the earlier work).

The statistical analysis using Cv was repeated using both Microsoft Excel (2010) and box and whisker graphs were drawn using Sigma Plot 12.5 (2013).

This approach using direct application for the testing and validation of the ATP devices was novel and this PhD study documents the first use of this method. The confluent application of the novel direct application method, and then the use of coefficient of variance for statistical analysis was a

comprehensive approach to the validation which allowed a uniform understanding to be developed on the reliability of the ATP devices across the various branded platforms.

One important finding through this comprehensive methodology (both the ATP analysis and the statistical methods) was that the outliers shown in the box and whisker plots were both above and below the 10th and 90th percentile whiskers. This was a regular event and not just a single time incident. This was observed with all of the ATP devices tested, and was observed in all of the tests including pure ATP and both bacterial species. There is no warning of this variability to users of the ATP testing devices. The methodological approach used in the 4th Whiteley paper is the only paper published which has demonstrated so clearly this level of unreliability.

Risk Analysis using FMEA (Whiteley paper 3)

The results from the first laboratory phase provided a more robust approach to the interpretation of the data arising from ATP testing. The uncontrolled variability in ATP test data requires careful consideration when preparing a sampling plan for cleanliness monitoring using ATP testing. The strong benefits of ATP testing such as the timeliness of a quantitative data reading has meant that research on using ATP testing within healthcare settings has continued to be widely published. The goal of using ATP to provide a quantitative basis for cleanliness monitoring and even the use other monitoring systems with an integrated approach to cleaning monitoring had been attempted.[9-11]

The early PhD work on risk with the posters etc., opened a pathway to reconsider the cleaning monitoring methods with the data that had been generated on the ATP testing and FM spot experiments. Clearly the risks around failure were more significant than the benefits alone, and so consideration was given to the appropriate risk assessment tool with which to consider the integration of ATP testing into a cleanliness monitoring process in conformity with the Australian requirements.

The Australian Commission on Safety and Quality in Healthcare produced a new framework in 2012 of 'National Safety and Quality Health Service Standards' (NSQHSS).[12] This introduced into Australian healthcare the requirement for a uniform approach to quality standards setting, quality improvement goals, and risk based management of hazards for patients and all stakeholders.

There are ten broadly focused "national safety and quality health service standards" within the NSQHSS. The third standard focuses on "preventing and controlling healthcare associated

infections". Under standard three, criteria 3.15 states that healthcare facilities should comply with the intention of a clean and hygienic environment by "Using risk management principles to implement systems that maintain a clean and hygienic environment for patients and healthcare workers".

The introduction of NQHSS standardised approach to quality assurance and risk assessment in principle provided an ideal backdrop for the application of a risk assessment based approach to examining each of the four cleaning monitoring methods.

A review of various methods of the risk assessment was undertaken with consideration of the significant reference document, which was ISO 31010 (IEC/FDIS) titled "Risk Management – Risk assessment techniques".[13] This comprehensive review document covers the 31 major techniques for risk assessment. The document also provides a ranking assessment for applicability. Two of the authors of the third Whiteley paper had experience with FMEA, but the process was also well rated for reliability and applicability for the intended task.

After reviewing the literature on risk assessment methods and tools, the decision was confirmed to use Failure Mode and Effects Analysis (FMEA) to examine all of the four major cleaning monitoring methods. FMEA is a prospective method that through an FMEA committee uses the known, described, documented or identified failure risks, and compares their individual impacts on the intended outcome. The FMEA method also then considers and recommends possible strategies to mitigate these impacts in practical settings. The FMEA process within industry settings frequently uses a 10 point rating scale, but so as to fit into the Australian quality framework which uses a three tier rating approach the method was adjusted to conform to the Australian NSQHSS approach to risk assessment.

The committee of three (authors) individually considered what would be the failure modes for each of the cleaning monitoring methods, and subsequently what would be the rating against the three scoring criteria used with FMEA including frequency, severity and detectability. The American Journal of Infection Control has a limit of 4000 words and a limit of just three graphs or tables, and so only the 15 high and medium failure modes could be included in the paper.

This paper is the first ever comprehensive assessment of the failure modes of each of the cleaning and cleanliness monitoring assessment methods. The application of this method allowed for the consideration of the combination of the four methods which took account of the failures but which also allowed the benefits to be respected.

The critically important recommendation arising from the study was the outline by Figure 1 in the FMEA paper (the 3rd Whiteley paper) which proposed a new way to combine all of the cleaning monitoring methods into a single integrated approach to cleanliness and cleaning monitoring. The recommendation on integration demonstrates that the strengths of each of the respective methods can be combined to overcome the weaknesses of the other methods. The use of FMEA as a method of assessment for cleanliness monitoring is novel and contextualises the work within the PhD on building a proposal to constructively resolve a new scientifically valid and integrated approach to cleanliness monitoring within healthcare settings.

The paper was submitted using this approach and has been viewed or down-loaded on more than 300 occasions with one citation so far.

Pilot Study Methods (Whiteley paper 5)

The fifth paper within the PhD study was a pilot study that applied the learning from the early ATP papers, and applied the recommended integrated method for cleanliness monitoring within a busy and fully operational Intensive Care Unit (ICU). In this case the cleanliness monitoring integrated three of the four methods into a singular approach.

The particular ICU was concerned about the potential for transmissions of healthcare associated infections (HAI). The project team for this study (co-authors) included staff from the School of Medicine, the Ingham Institute and Macquarie University. The project was approved by the ICU (Medical Director) and the project didn't require ethics approval (a survey of the environmental cleanliness not involving patients or staff is not included by the Western Sydney University ethics guidelines).

The three cleanliness monitoring methods used were visual inspection, rapid ATP testing and microbiological sampling of surfaces including growth on selected media. The methods used in the study are well described in the paper and the discussion here is intended to amplify the reasoning behind the approach taken in the study.

The comparison and first method used in the study was a sampling run using pre-determined lists of high touch objects (HTO). These HTO were identified from the literature. This first sampling run used a lone researcher and applied a novel sampling approach and re-growth based on selective media

using biofilm recovery principles.[14, 15] The sampling on this basis yielded a very low recovery rate for MRO of just 1/23.

The role of visual inspection is core to the Australian approach to the monitoring of both cleaning and cleanliness.[16, 17] Whilst the auditing approach taken in the reference documents rates various items on a checklist, the approach in this study was more observational and took account of items and surfaces that were seen to be frequently touched by staff in the ICU.

Notes were also taken on terminal cleaning efficiency following patient removal and methods of decontamination during terminal cleaning. Staff handwashing practices were observed but not audited. No feedback was provided to staff on either cleanliness or handwashing or touching items with gloved hands outside the immediate patient zones.

There are five separate findings arising from the study that are well discussed in the paper. The methodological approach on the second sampling day in particular had recognised flaws, but even despite this the application of the learning on ATP sampling proved a decisive benefit when combined with the visual component in providing almost immediate and immediacy of feedback on relative cleanliness of different surfaces. This mixed method approach allowed more targeted microbiological sampling which significantly enhanced the results in locating the presence of multi-drug-resistant-organisms (MRO).

The method of duplicate sampling using two different ATP testing devices was also highly novel and illustrated the risks of uncontrolled variability and a lack of a uniform scaling approach. The almost complete non-correlation of the two devices was another previously unpublished and highly novel outcome which is well discussed in the paper with reference to past literature. This was a particularly interesting finding given the overall results which demonstrated the benefit of using rapid ATP testing.

The statistical analysis used followed conventional methods. The use of chi-square adjusted for low sample numbers (with incorporation of the 'Yates' correction) and Fishers exact test indicated the significance of the core findings using this new integrated methodological approach to cleanliness monitoring. The low sample numbers did limit the potential for a realistic finding using a Receiver Operating Curve (ROC) to establish appropriate cleanliness thresholds for each of the devices and these were distinctly different numbers, albeit without comparative statistical significance.[18, 19]

The basic approach to finding biofilms in an ICU remains under development and only recently has the presence of dry surface biofilms been demonstrated within an ICU.[15, 20] The methods for reproducing biofilm growth in laboratory conditions are also in the early stages of development.[21]

The presence in ICU of dry surface biofilms which harbour MRO poses a profoundly increased hazard and elevated risk for the cross infection of already vulnerable patients.

The integrated method demonstrated in this paper showed improvements over all previous attempts for the identification of the locations of MRO which may be residential on hospital surfaces. This method also had a very significant outcome in indicating that the HTO that are of high risk for harbouring MRO are not just clustered on the near patient surfaces around the ICU patient bed.[22-24] By using the visual component of assessment to identify HTO, and then applying the rapid ATP testing to those surfaces, the real time effect directed the microbiological sampling to clinical workspace well out of the anticipated sampling locations around the near patient surrounds.

This new integrated method for cleanliness monitoring demonstrated that there was a 7 fold greater likelihood of quickly finding the less clean locations than using a simple HTO list approach which has been the dominant method in Australia and elsewhere. This exciting development has only been enabled by the new methods developed and trialled during this PhD.

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Chapter 3

A thematic & critical review of each of the papers included for PhD

Précis

This chapter provides a thorough review of each of the papers included in this PhD submission. The chapter outlines how the five papers together establish a cohesive thematic study which comprises this single body of original research. Each paper will be contextualised for contemporary relevance, methodology, results and contents, and the contribution of each of the papers into the single theme for this PhD thesis. The learning attained and the contribution of the principle author and contributions of the various co-authors in each of the five papers will also be outlined in this chapter. The papers are presented in chronological order.

Introduction

In the consideration of this PhD by a series of publications, there are five fully peer reviewed papers which have been published in scholarly journals. The five papers outline the validation of ATP testing devices and their application for cleanliness monitoring within healthcare settings. These five papers interlock as a continuous theme on the validation and use of rapid ATP testing. The study commences with laboratory work focussed on ATP device performance in a limited range of controlled experiments, and then moves onto statistical work focused on the reliability of the ATP testing devices. From here the theme of the study contextualises the use of rapid ATP testing as a cleanliness monitoring tool within healthcare settings, before coming back to the laboratory to more fully document the performance of the ATP devices using bacterial cultures replicative of healthcare applications. The final paper is a field based pilot study that uses two ATP testing devices that demonstrates a practical application of these devices within healthcare settings by building on both the risks of use and the validation process.

The first paper is the initial validation study published to reveal both useful and concerning findings on ATP measurement comparing three commercial brands of ATP devices.

The second paper develops the work of first paper, but applies the coefficient of variation (Cv) as a statistical measure of variance in the ATP readings obtained during the controlled experiments.

The third paper steps back from the benchtop work to consider the overlapping flaws of rapid ATP testing and the other accepted cleanliness monitoring methods used within healthcare settings. The study looked into the flaws of each cleaning monitoring method, and also useful synergies from the use of ATP testing with the other three major cleanliness monitoring tools. This third paper introduces to infection prevention professionals a new novel risk assessment approach using Failure Mode Effects Analysis (FMEA). A new and novel approach to cleaning/cleanliness monitoring is outlined in the paper.

The fourth paper extends the work of the first and second papers and investigates the statistical problems of uncontrolled variability using the commercially available ATP devices using both pure ATP and two representative bacteria which are commonly found within healthcare settings. The fourth paper applies Cv to establish the case for careful interpretation of data arising from field based use of ATP testing devices based on the validation studies.

The fifth paper takes two of the ATP testing devices out of the laboratory and uses the strengths of rapid ATP testing to overcome the weaknesses of environmental microbiology. This fifth paper demonstrates that rapid ATP testing, once validated, and despite imprecision and other flaws, can contribute practical usefulness in cleanliness monitoring and infection prevention within healthcare settings. This paper applies the teaching of papers 1, 2 and 4 (instrument dynamic range and limitations, variability and imprecision) and paper 3 (FMEA revised risk based approach) in a field based study within an active Intensive Care Unit.

First paper review

Authors: Whiteley GS, Derry C, Glasbey T.

Title: The comparative performance of three brands of portable ATP-bioluminometer intended for use in hospital infection control

Publication: Healthcare Infection: 2012:17:91-97

Impact Factor: Not available: CSIRO as Publisher indicates IF <1

Citations: 11 (Scopus search 4th February 2016)

The seminal paper by Griffiths et al., for rapid ATP testing within healthcare was published in 2000.[1] Griffiths applies his personal experience in using rapid ATP testing within the context of food manufacturing and plant hygiene and through this small field based study indicated that ATP testing would be highly suitable for a similar application in cleanliness monitoring within healthcare settings. The findings demonstrated that ATP testing was superior to both visual inspection and environmental microbiology as a cleanliness monitoring tool within a healthcare setting. What was surprising in the papers which followed was that the studies were undertaken without having conducted any validation process for the ATP testing devices used in the studies.

In 2011 a single 'concise communication' paper was published that attempted to provide a validation process for ATP testing against quantitated bacterial suspensions using Staphylococcus aureus.[3] The findings were that of the five commercial ATP devices tested, all of the ATP devices lacked robustness in quantitation, particularly at low bacterial concentrations.

Against that backdrop, in this first study, we set out to validate three commercially available ATP devices using a validation process in accordance with Pharmaceutical Industry guidelines.[4] This meant firstly looking for linearity of response and comparing the results to a 'gold standard' and fully validated reference test. For this purpose, an HPLC method was used as a comparison.

It was also recognised that testing against live bacterial cultures was also subject to the risks of variability in cellular lysis and ATP content. A new method was required, so in this first paper a pure (known source) of ATP was used and a standard volume (20µL) was applied directly to the ATP swabs using a validated micro-titre pipette. The advantage of this approach is that the experiments focused on only one measurement variable, which was the ATP reading (in RLU) from each device. This was a novel and previously unpublished method for validating these ATP devices.

The findings of the paper were also novel. This remains the first published study in the medical literature that has focused on validating the dynamic range and reading scale of commercial ATP

devices based on pure ATP, directly applied to the swabs, and thus free from other confounding variables (cellular lysis, swab absorption, ATP cellular density and others).

The results outlined in this first paper demonstrated that ATP devices could be relied upon when testing serial dilutions where concentrations were 10 fold differentiated as shown in Figure 1 within the published paper. The dynamic range of ATP readings (from lower limit of detection [LLD] to upper limit of detection [ULD]), was also shown to be different for different devices. The work also clearly demonstrated that at any quantitated application of ATP, each of the ATP devices showed a different scale on RLU.

This first paper also clearly demonstrated that the reading scale of the different branded ATP devices lacked any overlap and therefore had nil interoperability. This has dramatic implications on the prior published cleaning threshold values in RLU from studies with only one branded ATP device, which could not meaningfully be applied to any other ATP device. The recommended ATP cleanliness threshold of 100 RLU was clearly marked on Figure 1 in the paper to illustrate this problem.

However, the study also demonstrated that where the concentrations of serial dilutions were less well differentiated (we used 20%) then the ATP devices would reliably differentiate between the dilutions as the data irregularly overlapped as shown in figures 2-4 within the published paper. For each of these figures the 100 RLU cleanliness threshold was also marked illustrating how inappropriate this single level measurement was for the different branded ATP devices.

The single level cleanliness threshold was a major problem not previously identified in the literature. In fact several studies had accepted the suggested RLU cleanliness threshold from 500 RLU using a 3M branded device and this level of 500 RLU was reconfirmed in another study (2009) which also noted a lack of correlation between ATP/RLU results and microbiological results (cfu/mL)[1,5]. In another study the threshold value was recommended to be reduced from 500 RLU down to 250 RLU in an effect to improve the comparability with comparative results using environmental microbiology, and this was done using a 3M branded ATP device.[6]

A study published in 2009 by a separate group compared two different devices (3M and Hygiena) without doing any comparative validation and simply noted the difference in results.[7] Finally, another published study preferred the Hygiena branded ATP device and in the study seamlessly moved to recommend that the cleanliness threshold using ATP be reduced from 250 RLU (3M) down to 100 RLU (Hygiena), without any comment over the difference in scaling (interoperability) between the different branded ATP devices, thus assuming uniformity of scaling.[8]

In the same time period, a number of papers were published, or accepted for publication which posed questions over the validation of ATP for applications in healthcare cleanliness monitoring.[9-10]. Whilst the methods and commentary used were not based on the first paper method of direct application of ATP, a common issue identified was the lack of uniformity in scaling, the risks posed by uncontrolled confounders, and the lack of correlation with ATP data and contamination, particularly at the lower levels which was critical for healthcare applications.

Another notable finding was the identification of the differing abilities of the ATP testing devices to quantitate the ATP concentrations at both ends of the dynamic range (Figure 1 from the paper). Within healthcare settings the instrument performance at the upper limits of the dynamic range is of a lesser concern than performance towards the lower end of the dynamic range. At the lower end of the dynamic range, two of the ATP testing devices (3M and Kikkoman) demonstrated poor quantitation below 100 RLU and could not reliably demonstrate any reading below 30-50 RLU. The introduction of the concept of a Practical Zero was important for field usage of these ATP devices.[11]

In this first paper, the study results both supported and extended the findings of Aiken et al.[3] However, the paper also outlined through a controlled validation processes using only pure ATP, that the dynamic range of different brands of ATP testing devices was an unrecognised variable for studies using ATP testing. The problems of scaling, variability and imprecision required careful validation and consideration and particularly where measurements were attempting to distinguish between similarly soiled surfaces. The findings of the first paper were the foundation for the following work as part of the PhD study.

There were some limitations to this paper. Firstly, the data set was based solely on exposure to pure ATP and not to any bacterial species. Secondly, it was published in a local Australian journal which somewhat limited its global reach and limited the possible influence of the findings. Thirdly, only a single ATP device from each brand source was used, and consumables, whilst stored as per manufacturers' instructions, were not tested linearly across the time-line of recommended shelf life (to determine if the shelf life of the consumables is another variable).

In the peer review of this paper both of the peer reviewers commented that this research would enhance the discussion on environmental hygiene.[1] The first reviewer said that research when published "…*will add to the discussion around evaluation of environmental hygiene in healthcare and also provide direction for further research into this subject*". The second reviewer similarly

[1] email: official response from the editor dated 9th July 2012

commented "*This study may generate and lead to further discussion*…" and also that the research raised "*Valid concerns regarding the concept of a cleaning benchmark*…" which is precisely the outcome expected from the findings. There have been 9 citations so far of this paper.

The learning outcomes from this paper were substantial. Firstly, this was the first major authored paper and required substantial changes to my writing style. Secondly, the work itself reset my practices around experimental design, management of the resources (including time and co-authors) and also the recording and presentation of the results into a format suitable for publication.

The co-authors contributed through various of the practical aspects of the study, including Dr Glasbey in setting up, validating and working with the HPLC, and ensuring that the pure ATP remained stable for the period of the experiments. Dr Derry contributed in editorial assistance and also in the preparation of the statistical analysis including tests of association and reframing Figure 1 of the paper as used for the published version. The principle author was responsible for all re-writing and re-drafting following consultation with the co-authors, the recording, preparation and presentation of results including graphs, statistical analysis in consultation with Dr Derry, and primary responsibility for the submission and peer review of the paper.

Second paper review

Authors: Whiteley GS, Derry C, Glasbey T

Title: Reliability testing for portable adenosine triphosphate bioluminometers

Publication: Infection Control and Hospital Epidemiology: 2013:34:5:338-540

Impact Factor: 2015 IF: 4.15 5 Yr IF: 4.50 (Source: Research Gate: 4th February 2016)

Citations: 6 (Scopus search: 4th February 2016)

In this second study, the results from the first study, using only pure source ATP and testing against the three branded ATP devices, were examined using a variance measure. The paper was accepted for publication into a 'Special Topic' edition to coincide with a "SHEA Spring Conference" with the theme "The role of the environment in infection prevention". One of the peer reviewers commented[2] on the paper "*Overall, clearly written… I think that it [the paper] highlights a significant issue related to interpretation of results due to variability among different manufacturer products*", which was precisely the point of the findings.

The results from the first study showed an unexpected level of variance. This was particularly so given the carefully quantitated volumes of pure ATP applied to the different ATP device swabs, from the same ATP solution container, on the same day, in the same experimental round with quantitation confirmed using a validated HPLC which had demonstrated almost no variability. Whilst the first paper had demonstrated the dynamic range of the ATP devices, and their poor precision when using closely separated dilutions series (20%), the level of variance was considered in this second paper using a variance measure of Coefficient of Variation (Cv).

The Cv is a calculation of the standard deviation for an individual data set over the mean for the same data set. Cv is a simple expression of the variance and is the inverse of the signal to noise ratio. The paper set out the results from the calculations in graphical form in figure 1 as a scatter plot. In this paper all three of the ATP devices demonstrated a Cv as high as 0.4, i.e. the standard deviation for a data set was as high as 40% of the data mean. This level of imprecision is very poor and normally would be considered unacceptable for a scientific measurement device.

This was the first study to use Cv as a measure for variance with ATP testing devices. All of the brands performed equally poorly and none of the brands gave any indication to users as to the level of variability. This paper presented a new confounding factor for studies where ATP testing was used as a measure of cleanliness. Whilst a standardised level of ATP (RLU) may be set with any particular

[2] email: official response from the editor dated 28th October 2012

ATP device brand, the high and uncontrolled level of variance that is obtained in actual use of the ATP device, limits the meaningfulness of any RLU threshold due to device reading imprecision.

This paper added to the debate over the usefulness of ATP/RLU cut-offs/thresholds for setting cleanliness standards for infection prevention in environmental surfaces and has received a number of independent citations. The implications of this work in combination with the first paper have been to undermine any suggestions that a universal ATP/RLU cut-off or threshold could be used for cleanliness monitoring in the context of hospital infection control. There remains the need to develop better science around cleanliness monitoring but at the point of publication of this paper, more work was required to thoroughly validate the ATP testing systems.[12]

The limitations of this study were similar to that of the first study. Firstly, the work focused on application of pure ATP to the swabs rather than bacterial suspension or cultures. The second limitation was that although published in one of the key global journals in this infection prevention field, the paper was a "Research Brief" (word limit of 900 word plus one figure or table, and < 10 references), and this so was not a full-length research paper. Finally, this second paper did not attempt to assess whether ageing or storage of the consumables had any impact on the variability of ATP/RLU readings.

The learning experiences from the paper were highly productive. Firstly, the application of the Cv as a measure of variance provided a new platform from which to assess instrument reliability. It had been quite a confronting experience to find the unexpected variability and so separate statistical advice was sought from Mr Paul Fahey, who is acknowledged at the end of the paper. The authorship lessons from this paper were also that the strict limitation on word count required focus and acuity to ensure that the paper adequately described the work and expressed the implications for the findings. That the peer reviewers recognised this aspect was most pleasing.

Third paper review

Authors: Whiteley GS, Derry C, Glasbey T

Title: Failure analysis in the identification of synergies between cleaning monitoring methods

Publication: American Journal of Infection Control: 2015:43:147-153

Impact Factor: 2015 IF: 2.21 5 Yr IF: 2.50 (Source: Research Gate: 4th February 2016)

Citations: There have been 300+ downloads of the paper since publication but citations have not yet appeared in the literature.

Following the publication of the first two papers in 2012 and 2013, and a range of other critical publications on the appropriateness of ATP testing in cleanliness monitoring, a re-evaluation of the project was required. The clear advantages of rapid ATP testing were still present, but were now offset by the findings of the first two papers and several other relevant publications. One significant paper, which went on-line in 2012 (Sciortino et al.), proposed to have achieved validation of three commercial ATP devices.[13] However, even the singing endorsement of ATP testing by Sciortino et al., was subjected to pointed criticism over the poor response of the ATP devices at the LLD and a number of other confounders.[14]

The need for a suitable audit tool for cleanliness monitoring and cleaning validation had already been established and the practicality of rapid ATP testing recognised.[15,16] A second important cleaning verification tool that had become available from 2006 was the Fluorescent Marker (FM) invented by Dr Phillip Carling. This innovation used a small dab of polysaccharide based glue, laden with fluorescing material that dried to a clear film (still clearly visible under small low power UV light) but which could be wiped off easily through normal cleaning processes.[17] Because the film is thin and clear the removal of the FM became an easy way to assess if a surface or object had been subjected to the intended cleaning (wiping) process. A broad array of participating hospitals took part in a large validation trial and the results indicated that this tool not only improved the rate of cleaning but also appeared contributed to a diminishment to overall recovery rates of microbes responsible for HAI.[18]

Some laboratory work was conducted on two forms of the FM which were supplied directly by Dr Carling. The first was the original “dab-on” solution. The second was the licenced/commercialised version under the tradename “DAZO” from Ecolab (Minneapolis-St Paul, MN). Laboratory studies as part of the PhD project considered drying characteristics and visibility. The results from those studies

were contained in several posters but as these findings only reconfirmed the literature the work was not submitted for publication.

Several pertinent studies verified the usefulness of FM including one in Australia and another study where the technology was used in tandem with ATP testing.[19,20] FM was also found to be an ideal tool for cleaning staff feedback and education, but it could also be misleading when cleaning staff could themselves identify the FM spot locations and then selectively clean and remove just the FM spots.[21-23] Even by itself the results of FM still can lag the Hawthorne effect of personal observation.[24]

The substantial observation on FM was that its use within healthcare settings was a constructive way of monitoring surface wiping and therefore reducing bioburden. Whilst FM does not provide any quantitative assessment of surface cleanliness per se, it does reflect that surface cleaning has been attempted and thus is a qualitative measure of cleaning (staff) efficiency. Studies using FM, ATP and studies using both FM and ATP have all demonstrated the usefulness of timely feedback to cleaning staff through these new cleaning monitoring methods.[25-27]

However, there was still no credible alternative cleanliness monitoring method that provided all of the requisite information to provide an adequate level of quality assurance over environmental surface cleanliness within healthcare settings. There remain only four practical options for cleanliness monitoring and cleaning validation which are all reviewed in the paper. The four cleaning monitoring methods are visual inspection, microbial recovery, ATP testing and FM technologies. Each of the methods has advantages, but equally all of the methods have shortcomings that limit their practical usefulness for cleaning auditing as an aid in infection prevention.

This third paper is a study that steps back from the overlapping and sometimes conflicting array of advantages and disadvantages of the different cleaning and cleanliness monitoring methods. The goal of the study was to conduct a structured risk assessment on the proposition for using ATP testing within the broadest context of cleaning monitoring and compare the risks associated with ATP testing with the three risks associated with the three other cleaning monitoring methods.

A structured risk based approach using Failure Mode and Effects Analysis (FMEA) was used by our small team to reflect on the data from the earlier studies, and from the broader and developing infection control literature. The FMEA team then assessed the failure risks of each of the cleaning/cleanliness monitoring methods. FMEA allows for the interrogation of potential or recognised failures for use each of the methods of cleaning/cleanliness monitoring and to both grade and attempt to mitigate each failure mode.

Only one other reference using FMEA in this field could be located, and it was for a poster presentation only and no paper was published.[41] Therefore, this third paper is the first of its kind published in the infection control literature.

The study in this third paper, was an important breakthrough in novel thinking on how to integrate the cleaning/cleanliness monitoring methods and unlock potential synergies between the various methods. Figure 1 in the published paper proposes a new pathway forward using all of the available cleaning/cleanliness monitoring methods with a scientifically based, integrated assessment of cleaning and cleanliness on hospital surfaces. In all prior publications authors have tended to recommend their pet/partisan method over and above the other methods. Whereas using FMEA to structure the risk assessment, this study arrived at a recommendation for process improvement that contextualised the use of ATP testing, as well as recognising the advantages of visual inspection without the need to denigrate visual inspection as an inferior monitoring method.

The Australian requirements for a three-tiered risk classification were followed in the paper to provide a simple and locally flavoured outcome.[28] FMEA also assesses and grades each failure mode for its likely frequency and the severity of the failure, and combines these two criteria with an assessment of the detectability of the failure mode to arrive at an overall risk quotient. The scoring approach reflected the Australian requirement for a three tier scoring scheme (graded 1 to 3) which once the risk quotient was obtained allowed each failure mode to be then grouped into risk classifications. The risk classification approach used in the paper separates the failure risk for each of the failure modes for each of the cleaning monitoring methods. In the paper, only the worst case failure modes are shown in Table 2 of the published paper.

This novel approach to risk assessment for the cleaning and/or cleanliness monitoring methods had several limitations. Firstly, it was a small team used for the study and only two of the authors had previous experience with the FMEA methodology. As a small team care had to be taken to ensure the findings were anchored in objective literature and not just a circular and biased selection of failure modes. Secondly, as a novel approach it was initially difficult to secure peer review acceptance with reviewers who were unfamiliar with risk assessment strategies and methods, which tend to be predictive and qualitative. Finally, the small team did not use any practising healthcare professionals nor anyone currently engaged in an infection prevention role.

This was a challenging paper to write. One of the peer reviewers commented "…*the use of one monitoring technique to supplement another monitoring technique is new. Also, the use of FMEA for the purposes of evaluation is new and provides food for thought for the infection preventionists and*

for environmental services."[3] This was an encouraging outcome after the effort applied in taking FMEA as a medical devices standard risk method and applying it in the assessment of methods with products, but for the common purpose of cleaning/cleanliness monitoring.

In the process of this study and the writing of this paper a large number of lessons were learned. Whilst the outcome of the findings was pleasing, the process taught the importance of perseverance and the value of finishing the task. The management of the team process with the risk of selection bias in findings required additional rigour and reflection. The peer review process for this paper required major reconstruction of the paper and the key findings shown in Table 2, so as to bring the paper back to an acceptable size and format for the journal. The entire co-author/team group participated in the FMEA process, but I was the principle author in every aspect. Dr Derry also assisted greatly in the framing of Figure 1.

This paper has been downloaded on more than 300 separate occasions since publication in February of 2015, and citations are expected. The full influence of this paper will be on-going and an abstract for an oral presentation is being submitted for the next meeting of the American Practitioners of Infection Control conference in 2016. The American Journal of Infection Control is recognised globally as the number one ranked Nursing journal on Infection Control and has an enormous readership.

[3] email: official response from the editor dated 25 September 2014

Fourth paper review

Authors: Whiteley GS, Derry C, Glasbey T, Fahey P

Title: The perennial problem of variability in adenosine triphosphate (ATP) tests for hygiene monitoring within healthcare settings

Publication: Infection Control and Hospital Epidemiology: 2015:36:6:658-663

Impact Factor: 2015 IF: 4.15 5 Yr IF: 4.50 (Source: Research Gate: 4th February 2016)

Citations: Publication was June 2015. No citations at this time.

The fourth paper extended the work of the first and second paper, attempting to overcome some of the shortcomings of the earlier papers by broadening the work validating the ATP devices beyond pure ATP into bacterial responses. The methods used followed the teaching of the first paper in that the materials were applied directly onto the ATP swabs in order to remove variability arising from swab materials or swabbing technique. The use of different bacterial species opened up the issue of cellular lysis as a variable but the use of multiple units and quadruplicate applications minimised the impact of this source of variability.

The work in the study consisted of a range of dilution series, both 10-fold and 20% dilution series. The freshly prepared bacteria suspensions were provided but all dilution series, including preparation of materials, confirmation of validation for pipettes, dilution series, application of the diluted suspension, enumeration and confirmation of enumeration, recording and interpretation of results, and work area clean up were all conducted by the principle author under both direct and delegated supervision within a PC2 Microbiology Laboratory.

The purpose of the series experiments was in the first instance to investigate and reconfirm the dynamic range of each of the ATP devices using both two bacterial species and a pure ATP solution. Quantitation of the ATP was again confirmed using a validated HPLC method. Learning from the earlier work, nearly every individual dilution sample was tested in quadruplicate with each of the ATP devices. The benefit of this was that the Cv could again be calculated for the entire sample group and thus express clearly the difficulty of this uncontrolled variability in the use of ATP devices.

This paper has the benefit of further time to reflect more appropriately the breadth of literature that has been published in relation to ATP testing. Even during the preparation of this fourth paper, a separate study was published (manufacturer funded), using just one branded ATP device and that study not only failed to reflect on the critical literature in the field, but used a single point sampling methodological approach.[29] A letter to the editor (principle author GS Whiteley) was written and submitted to Infection Control and Hospital Epidemiology criticising the sampling method, and this

letter was published by the journal.[30] What was interesting in the reply was the concession by Visrodia et al., that the work would have benefited from a better sampling method, but they were concerned over the "cost" implications for field work applications.[31]

This was the perfect introduction to the importance of the extension of the earlier work and the new paper submitted. One of the peer reviewers appropriately commented as follows: "*This important report represents an in-depth extension of prior studies by the authors which increases both the depth and breadth of analysis of practical aspects of the use of ATP bioluminometers which had been widely marked for use in evaluating the cleanliness of near-patient surfaces in healthcare settings. Although concerns regarding the accuracy and sensitivity of ATP testing in clinical settings have been repeatedly raised during the past five years, this report represents the first in vitro analysis to objectively and independently (not manufacturer sponsored) assess the accuracy of the ATP systems currently being used…The methods of evaluation were innovative and appropriate. The use of co-efficient variation (Cv) was particularly appropriate.*"[4]

The work itself both extended the findings of the earlier work, but also introduced a fourth and again distinctly differentiated ATP device (Charm). As this paper was a full research submission the expression of the data could also be amended to better reflect the data sets covering the variability found with testing against pure ATP and the two bacteria (*Pseudomonas aeruginosa* and *Staphylococcus epidermidis*). The analysis of the data was conducted using Cv and as part of the statistical analysis the decision was taken to express the spread of the Cv data using box and whisker plots rather than the dot plot format. The box and whisker format allowed a more simplified presentation on the spread of the data and also showed outliers.

Each of the ATP devices demonstrated similar patterns of variability which are shown as box and whisker plots as shown in Figures 1 to 3 in the published paper. An important observation was that outliers were present in the data as outliers, both above and below the whiskers which were placed at the 10th and 90th percentile calculations for each device and against each test solution. This has major implications for sampling methods and perfectly illustrated the point which was made in the earlier letter to the editor.[30] Figure 4 presented another aspect to the data which simply expressed the range and median points for the range of Cv for each device in each test platform. Whilst it looks like the ATP devices could be ranked for performance, in fact the key finding is that every one of the branded ATP devices showed a Cv range above 0.4 which means that for any reading obtained in the field, there is a 20% possibility that the reading could be wrong by a factor of 2.

[4] email: official response from the editor dated 3rd January 2015

This is a highly important finding and suggests an explanation for the poor precision of the ATP device when tested using a 20% dilution series (first paper). This finding also underscores the importance of getting the sampling methods correct. A quadruplicate sampling approach with calculation of the arithmetic median will provide the most reliable pathway to flattening out the uncontrolled variability in ATP data obtained through sampling in the field. This is not always possible in field work, but there are other ways being considered in fresh research that could provide an alternative approach that would be equally suitable.

This fourth article had some limitations. Firstly, the work on dynamic range which compares the response to ATP and to two bacterial species could not be included in a single paper. Secondly, only one ATP device per brand was available for use in the experiments. Thirdly, the data set again could not accommodate measures that assessed variability across the entire shelf life period of the consumables.

A major benefit of the journal chosen (Infection Control and Hospital Epidemiology) is that the primary readership are clinicians who are the members of the Society of Healthcare Epidemiology of America (SHEA). Predominantly the membership is comprised of practising infectious diseases physicians. The journal also has a broad global readership amongst infectious diseases clinicians and other infection prevention professionals.

The full impact of this paper will continue for some time. But in response to this paper, one of the manufacturers of ATP devices has already responded with a letter to the editor.[32] This letter is from a marketing manager and strongly puts a case in favour of the use of ATP devices for cleanliness monitoring within healthcare settings, citing much of the literature already published in support of that view. The response from Whiteley et al., author group will agree with the basic proposition that ATP has an important place in healthcare settings, as indicated in paper 3, but that the issue of uncontrolled variability does require an active response from manufacturers. This variability issue is a different problem from attempting to correlate ATP readings with just bacterial contamination. [33]

Fifth paper review

Authors: Whiteley GS, Knight JL, Derry CW, Jensen SO, Vickery K, Gosbell IB

Title: A pilot study into locating the bad bugs in a busy intensive care unit

Publication: American Journal Infection Control: 2015:43:1270-1275

Impact Factor: 2015 IF: 2.21 5 Yr IF: 2.50 (Source: Research Gate: 4th February 2016)

Citations: Publication was June 2015. No citations at this time.

This pilot study took key learnings from the first four papers and applied them within the challenging environment of an active Intensive Care Unit (ICU) in a primary care hospital. The context for the study was the attempt to locate dry surface biofilms.[34, 35] The second author on this paper (Jessica Knight) had been tasked with the application of a new and more aggressive environmental recovery approach in an attempt to locate the presence of MRO and the possible locations of dry surface biofilms.[36] The first run of sampling was applied to high touch object (HTO) locations that the prior literature had indicated were potential reservoirs. Only a single sample from 23 swabs demonstrated growth of an MRO.

After discussions amongst the co-authors, it was agreed to trial the use of ATP testing as a rapid, real-time and relative indicator of surface cleanliness levels. It was hoped the ATP testing would discriminate between what was considered a clean surface and what may be indicated as a soiled surface and potentially contaminated. The sampling was somewhat *ad hoc*, and the methodology at risk of selection bias as was clearly indicated in the paper. The use of ATP testing did assist in directing the sampling locations towards the more contaminated surfaces in and around the clinical work area as shown in Figure 1 in the published paper. A significantly greater amount of samples demonstrated the presence of MRO once ATP testing was included in the field sampling and particularly on HTO and surfaces within the clinical work station as indicated in Table 2 in the published paper. Unfortunately as a Pilot study the number of samples was limited, and access into the working ICU was even more tenuous, thus preventing the extension of the work to extend the sampling and expand the number of surfaces to be examined.

The importance of this paper is demonstrated by the five separate important findings revealed in the results of the study. Firstly, the field testing reconfirmed that the two ATP devices used provided readings in RLU on a different scale which practically demonstrated the lack of instrument interoperability. Secondly and surprisingly these two sets of readings in a real life application had almost no correlation (R^2=0.0144), thus any comparison between the two sets of RLU readings could not be accomplished. Both of these findings are shown in Figure 2 of the paper.

Thirdly, the ATP testing gave the expected rapid outcomes which did aid in assessing the relative cleanliness of the different areas. As the results were quick and several "trial" readings were taken in the clinical work station, the actual readings were higher than for the other surfaces in the immediate patient zone (using the Hygiena ATP device), thus indicating a relatively more unclean set of surfaces. The readings were separated in some cases by an order of magnitude on the RLU scale which satisfied the concerns on where readings were differentiated by less than a factor of two (4th paper) and as per the dynamic range findings (1st paper). The real time response of the ATP testing demonstrated that the strength of the ATP testing (instantaneous results) helped to overcome the weakness of the microbial recovery i.e. real-time delay between testing and results.

Fourthly, the statistically significant finding of the paper was that the use of ATP testing did assist in finding more MRO through environmental microbial recovery alone by providing the immediacy of feedback on surfaces sampled. Broadly the results were 1/23 on sampling without the assistance of ATP testing, and 12/36 with the assistance of ATP testing (using a matched sampling approach). As discussed in the paper, it was the very low recovery number (just 1 MRO positive sample) on the first sampling round that grounded the statistical findings. Another part of the reason for this may have been bias with the introduction of a second more experienced operator (the principle author), and this was also noted as a limitation on the paper.

The fifth and final finding was the significantly large number of MRO recovered from surfaces within the clinical work area (13 separate MRO locations). We are not aware of any similar findings, using any methods, of as many MRO recovered from an ICU in a single study in such a short period of time, although recent evidence supports a more aggressive approach to investigation.[37] This aspect of the work is continuing to be actively researched under a separate and on-going series of linked studies by several of the co-authors.

The peer review comments on the paper were emphatically positive.[5] Said the first reviewer: "*This was an exceptionally well written study with a tremendous amount of promise for MRO in the ICU. The study had a strong structure, analysis of results, and appropriate identification of strengths/ weaknesses.*"

The second reviewer said "*This was a very well planned and executed study, pertinent and timely. The novel approach to detecting MRO outside of patient care areas can provide infection control practitioners with another tool to identify and correct otherwise undetected sources of nosocomial infection. The statistical tools used were clearly described and appropriate to the study*."

[5] email: official response from the editor dated 22nd June 2015

This was the strongest set of peer reviewer comments of any of the five papers and sets a ringing endorsement to the work. The other pleasing thing about this feedback is that it included the lead up work of the earlier publications and provides a positive platform on which to use ATP testing.

One of the challenges arising from this focused series of studies on validation of ATP testing devices has been remaining positive when there were so many findings that indicate difficulties in working with ATP testing devices. This fifth paper was another deeply rewarding experience as it allowed the ATP devices to be used in a valid and significant way. This study took advantage of the advantages of ATP testing, and allowed to the problems in interpretation and disadvantages of the RLU relative reading scale to be cast in context and not overstated.

As the primary author my role in the research field work used skills acquired over my working lifetime, including extending my (long-ago) undergraduate training as an environmental health officer in observing the real aspects of field work in the ICU. Whilst my first co-author (Jessica Knight) did the microbial sampling I was able to conduct all of the matched ATP testing. As the principle author I also did all of the statistical analysis, although due thanks to Dr Chris Derry for his assistance in setting up the chi-square framework and revision based on the Yates correction due to the low sample numbers. The level of analysis in this 'pilot study' was more detailed and varied than any of the earlier papers and has greatly extended my analytical and statistical skill. Thanks also to Associate Professor Karen Vickery on the suggestions to run Fishers Exact test as a second tier and confirmatory method in addition to the chi-square.

The study limitations were very well covered in the paper, but it is appropriate to add some commentary on the implications of the findings as part of the PhD theme.

Firstly, a number of other studies have used predicative models including receiver operating curves (ROC) in justification of suggested hygiene thresholds that could be applied to ATP testing.[8,38,39] Each of these attempts at a single standardised RLU hygiene threshold have been based on a single branded ATP device with the assumption on the uniformity of scaling using RLU. This fifth paper is the first since Anderson et al., (2009) that used two differently branded/ scaled devices in a field study, and which appropriately expressed the quite distinct scaling in both the application and findings.[6] In this fifth paper the use of an ROC method suggested two distinctly different RLU thresholds (20 RLU: Hygiena and 350 RLU: Kikkoman), although neither threshold was correlated with MRO recovery.

Secondly, the findings whilst statistically significant in assisting with the locations of appropriate sampling locations within the ICU favoured no particular brand of ATP testing device. This too was an important outcome for the study.

Thirdly, this fifth paper provided an appropriate thematic close to the PhD as a single unit of study. This paper used the findings of all of the earlier papers and demonstrated the findings and learning gained through the study to finalise this single piece of work. Rather than using the ATP testing devices as a regular monitoring tool where the data indicates poor performance, this paper used the ATP tests as a one off method of determining the relative cleanliness of different surfaces for the purpose of environmental microbial recovery. The relative difference in cleanliness in the study was indicated by more than ten-fold differences in the RLU readings which our earlier data clearly validated as a strength.

The risks of single point sampling were overcome through the method of use, and through using a second ATP testing device as an alternative reading value. Whilst this had its own limitations, such as no discernible correlation between the two devices, the devices themselves both were able to indicate higher values for the more highly soiled surfaces.

The fifth paper was published in the December 2015 edition of the American Journal of Infection Control and was given the prominence of the lead article for that edition of the journal. A separate press release on the study and its implications for cleanliness monitoring and infection prevention more generally was authorised and circulated by the American Practitioners of Infection Control for general media. An accompanying Australian press release was also prepared by Western Sydney University to coincide with the publication.

The PhD Theme summarised

The project title for the PhD study is "A study into the validation of ATP testing devices for integrated cleanliness monitoring within healthcare settings". The work commenced with a range of experiments focused broadly on the validation of three commercial brands of rapid ATP testing devices. At the time of commencement of the laboratory work, there were a number of published studies suggesting that ATP testing could be used as an alternative to microbial recovery as a benchmark for cleanliness and hygiene standards within healthcare settings. Prior to this body of work, no independent study had been published validating the ATP devices using a method based on standardised industry technical validation guidelines.

The first paper demonstrated problems with the devices that required additional studies and illustrated the need for a full validation process prior to any broad acceptance of ATP testing. The second paper focused on the difficulties with data precision, which were demonstrated statistically using Cv, albeit on a study platform using only a known source pure ATP. The third paper continued the validation theme by examining the place that ATP testing had amongst the range of cleaning monitoring methods. This third paper contextualised both the place of ATP testing in cleaning monitoring and mitigated the weaknesses identified through the laboratory studies.

The fourth paper extended the work on the first and second papers with a greater array of validation directed experiments and accentuated the expression of the imprecision of the commercial ATP devices through the use of Cv as an already accepted and appropriate statistical platform. This work using bacterial suspension as well as ATP, and building on the earlier work with ATP alone, clearly outlined for the healthcare sector the difficulty of measurements using the current generation of ATP testing devices. The problems with ATP testing have been broadly recognised.[40]

The fifth and final paper concluded the thematic approach of the study and demonstrated that with a constructed approach to field use of ATP testing, and to interpretation of the data, the ATP devices can play an important role in monitoring of cleanliness and cleaning within healthcare settings. This was the goal of the study. The PhD thesis has demonstrated that the validation of the ATP testing devices is possible even with the limitations of the ATP testing devices revealed through these studies. The thesis and series of published findings has also shown that using ATP testing within an integrated cleanliness monitoring method provides a substantially improved approach versus the conventional methods. This has tremendous benefits for infection prevention across the entire global healthcare system.

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Chapter 4

Conclusions and Recommendations

Précis

This PhD set out to validate the use of rapid ATP testing for use in healthcare settings, and to integrate the use of ATP testing into an improved approach to cleanliness monitoring. This chapter draws the conclusions from the study arising from the findings of the five published papers. This chapter also comments on the apparent and remaining gaps in the knowledge and recommends the pathway for further research into this topical subject.

Conclusions arising from the study

The initial goal was to validate the use of rapid ATP testing for applications in cleanliness monitoring within healthcare settings. The study and publications of this PhD are the most comprehensive set of independent studies to date in the field of ATP testing, and have the advantage of comparing across a number of branded ATP testing devices. The studies incorporate a number of novel methods whilst following a standard industry process of instrument validation.

The major conclusions arising from this PhD are summarised below.

Findings from the laboratory validation phase of the research

1. The reading scale for rapid ATP testing uses relative light units (RLU). Unfortunately the scale is relative in every sense and no two meters tested use the same algorithm to arrive at a common reading or RLU level. This leads to a number of subset problems including:

    a. The absence of a standardised scale means that none of the branded devices provide a data set which is in common with any other branded ATP testing device;
    b. The lack of a common scale removes the capacity to calibrate the ATP testing devices on a uniform basis, thus it is not possible to achieve accuracy in the measurements in the normal scientific manner;
    c. The lack of a common RLU scaling leads to interoperability between differently branded ATP testing devices;

d. The readings from different units appear so totally dissimilar that no comparison is possible from individual readings between differently branded ATP testing devices;
e. The scaling is relative and so product innovation includes in one case additional reactivity (Kikkoman reacts to AMP as well as ATP) and in another the scaling algorithm includes a first step of at least three logs for a baseline (Charm). The impact of this is to further disassociate the commonality of RLU scaling;
f. The different ATP testing devices have different dynamic ranges when tested against quantitated and pure ATP (Figure 1, from the first of the published papers);
g. The limits of quantitation (lower and upper) for each of the ATP testing devices are reached without any indication given to instrument users;
h. The poor reading precision at the lowest part of the dynamic range for both the 3M and Kikkoman branded ATP testing devices leads to a practical zero point around 100 RLU. Readings below this level are relatively meaningless as the readings are below the lower limit of quantitation. The Hygiena ATP testing device demonstrated better reliability at the lower end of the dynamic range and showed a lower limit of quantitation at the indicated "0" (zero) point on the instrument. The Charm ATP testing device results are not shown in the included papers but that branded device appeared to have a lower limit of quantitation above 1000 RLU on its reading scale;
i. All four of the ATP testing devices have poor precision in RLU readings across their full dynamic ranges;
j. And, none of these problems is clear to any user without having done their own comprehensive validation.

2. Rapid ATP testing devices are capable of distinguishing between cleanliness levels that are differentiated by a ten-fold difference in underlying ATP levels. Figure 1 of the first paper demonstrates that each of the ATP devices was reliable in this measurement criterion when using a median reading approach. This has important and practical implications for reliability in the interpretation of field based readings from surfaces to distinguish between strongly differentiated levels of organic soiling on various surfaces.

3. The ATP testing devices have a poor capacity to distinguish between relatively similar levels of organic soiling is poor due to imprecision (Figures 2 -4 from the first paper). The poor precision with apparently uncontrolled variability in RLU readings suggests that the commercially available rapid ATP testing devices are generally not suitable for routine

testing against any specific cleanliness threshold expressed in RLU, and particularly where the surfaces have similar before and after levels of underlying ATP (cellular soils) contamination.

4. The cleanliness thresholds that have been suggested in publications as suitable for ATP testing, imply that there is a single and consolidated scale which is universally applied to all branded ATP testing devices, which is incorrect. A single RLU cleanliness threshold is not possible as each branded device has its own unique scale without interoperability. The first cleanliness threshold readings on the RLU scale were 500 RLU and 250 RLU, based on work using the 3M device.[1,2] The more recent 100 RLU was based on the Hygiena device.[3]

5. The uncontrolled variability in readings is not recognisable by normal users who are not trained in the interpretation of data arising from ATP testing devices. This variability is the same whether testing pure ATP or bacterial cultures. The variability is inherent within the rapid ATP testing systems (see Figures 1-4, fourth published paper). Thus, significant care is required prior to any sampling planning, and further care is required for the interpretation of any RLU data from field usage of ATP testing devices.

6. The high variability can be demonstrated using the statistical method of the coefficient of variance (Cv) as demonstrated in the fourth published paper. This statistical approach has allowed all of the discreet test points and samples at those points to be normalised for comparison purposes.

7. The Cv for each of the branded ATP devices tested, regularly reached above 0.4 meaning the standard deviation was frequently above 40% of the sample mean (see Figure 4, fourth Whiteley paper). At this level, there is a 20% chance that any reading will be inaccurate by a factor of 2. In practical terms, this implies that any individual reading should be regarded as subject to an error level of from 50% to 200% of the original reading.

8. The frequency of outlier results, both above and below the 10^{th} and 90^{th} percentile, both in sample values and Cv, strongly suggests that any sampling methodology should be based around the use of medians and not means due the likelihood of asymmetrical distribution within any data set.

Findings from the FMEA study

9. ATP testing rapidly indicates surface cleanliness whereas FM spot removal indicates the performance of a cleaning task and the overall achievement level in surface wiping by a cleaning process. Thus ATP is an efficacy measurement and FM is an efficiency measurement.

10. FM has the advantage of very rapid feedback to cleaning staff on cleaning efficiency. None of the other three cleaning monitoring methods (ATP testing, visual inspection or microbiological sampling) indicated cleaning efficiency as reliably as FM usage.

11. ATP testing provides a rapid digital readout from measurement of the residual biological soiling levels (cleanliness) of a surface. The speed of feedback on relative cleanliness by ATP testing provides advantages over the alternative cleaning and cleanliness monitoring methods (FM, visual inspection and surface microbiology).

12. ATP testing can be integrated into a cleanliness and cleaning monitoring with each of the other three cleaning and cleanliness monitoring methods to provide a better quality assurance outcome (see Figure 1, third published paper). This integrated approach uses the relative strengths of the different monitoring methods, to overcome the inherent weaknesses in each of the same monitoring methods. An example is that ATP testing can be used to guide microbial sampling by providing rapid feedback on relative surface cleanliness.

Findings from the field application of ATP testing within an integrated cleanliness monitoring process

13. The use of ATP testing to guide microbial sampling is significantly more likely to result in confirmation of contaminated areas as opposed to a list based approach or visual inspection alone.

14. The integrated methodology as per Figure 1 in the third published paper, was shown to be superior to using visual inspection or a list based approach in isolation when looking to identify less clean surfaces on which MRO may be coincidentally present on HTO or surfaces.

15. This active and integrated approach to cleanliness monitoring provides an improved understanding of the spread of soils and level or risk of transmission of infective organisms across both patient and non-patient/clinical zones within hospital ICUs. Elimination of sites where MRO are present is an evidenced based application of cleanliness standards that should contribute to reducing the risks of HAI.

16. The use of ATP testing was validated in accordance with the goals for the study. Despite the findings on precision and reliability, the use of an integrated monitoring methodology using the advantages of ATP testing resulted in findings which suggest that ATP testing can be used within healthcare settings for the purpose of integrated cleanliness monitoring.

Additional notes on practical ATP Testing for use within a Healthcare setting

17. A well planned study within a healthcare setting using ATP testing should be based on the following elements to enable the most representative results:

a. The ATP testing device and consumables should be well understood and consumables to be used should be within the sue by dates;
b. The High Touch Objects or surfaces (HTO)to be sampled using ATP testing should be selected and a sampling plan arranged that takes account of cleaning schedules and cleaning materials being used. Not every HTO needs to be tested in a sampling plan, but a selection of HTO should be tested on each sampling run, and if possible the HTOs tested should be randomised or varied between each sampling run;
c. Each site to be subjected to ATP testing should be initially tested, cleaned thoroughly, and then re-tested to ensure that the expected “best outcome” is established prior to any application of ATP testing within healthcare settings;
d. The sampling plan and frequency should take account of the budget for the cleaning monitoring processes using ATP testing, including the labour time;
e. Each surface should be sampled in duplicate (at least) and the sampling area should be the same amount of area for each duplicate sample. As note in this thesis, outliers can occur both above and below the median for any single reading and the possibility exists for a Cv >0.4 resulting in a 20% chance that any individual reading could be wrong by a factor of two. Consequently, if the duplicate samples are found to be very dissimilar (say separated by a 10-fold difference in RLU), then a third or

even fourth sample might be required. The median should always be preferred to an average result;

f. The sampling plan for any study should commence with an initial period that defines the 'normal' or 'expected' cleanliness level across the HTO sampled;
g. A second stage might involve an intervention which could include a new method of cleaning, or cleaning frequency, and/or staff feedback on the findings from the initial and subsequent sampling runs;
h. A final stage might involve a follow up sampling run to determine the success or sustainability of the intervention and may confirm the need for continued random monitoring to sustain any improvements in cleanliness;
i. Statistical models should follow standard pathways, although duplicate samples should use the median approach as the true reading.

Recommendations on future testing and investigation

This PhD study has opened a pathway for further investigation on a number of aspects of rapid ATP testing. These are not limited to the following suggestions but these proposals may form the basis of additional and subsequent research projects.

There several identified key project areas, which are summarised in the following paragraphs.

Researching a new scale for ATP testing devices

18. This project into a new and universal scale for ATP testing devices would include the following issues:

a. The RLU scaling approach is not standardised against a quantitative mass of ATP. This problem could be rectified, but there has been no suggestions from any of the commercial organisations to replace the subjective RLU scale with considerations of a suitable scientific approach to a new standardised scale for the expression of ATP reactivity;
b. The various manufacturers of ATP testing devices are in commercial competition with each other. Therefore, care will be required to avoid any suggestions of anticompetitive conduct or collusion between competitors, even for the desirable

scientific goal of a commonly agreed scale for ATP testing to replace the current RLU scales.

c. An independent and non-commercial organisation may be best suited to facilitate this outcome through a collaborative research program and dialogue with the ATP device manufacturers;
d. The testing conducted within this PhD demonstrated that a common reaction drives the response across the dynamic range (Figure 1, first published paper). This rate of response is seen to be on a common basis for three of the branded ATP testing devices. This common response to ATP across the dynamic range should be also investigated with respect to bacterial suspension cultures so as to confirm the dose-response curve for each branded ATP testing device.
e. The investigation of the new scale could be initiated by plotting the dose-response to ATP on a ten-fold dilution scale for each device. The slope of the line could be calculated and the data for each device mathematically converted onto a normalised scale to provide a new and quantitative scale for measurement of ATP.
f. The range of the ATP scaling could be reduced by recalculating the readings into the $\log_{10}$ for each number. Thus the variability that is so apparent on the existing scale is also flattened out when reduced to its $\log_{10}$ base value.
g. The new scale would need to reduce this variability and provide a sufficiently discreet distance between each value in the scale that overlap of readings is reduced thus improving the precision of each discreet reading point on the new scale. The scaling will also therefore likely reduce the range of the numerical scale between the lower and upper ends of the dynamic range.
h. It has been noted that the lower limit of detection for ATP testing devices when testing against a suspension culture of bacteria is between 10 up to 100 cfu/mL.[4] However, this will vary by organism possibly due to differences in lysis capacity.[5] The most preferential approach would be to conduct a validation process for a new scale using a pure and validated source of ATP, and then to cross check the device sensitivity with a panel of suitable bacteria.
i. The most important issue for any new ATP reading scale will be the need for improved precision at the lower end of the dynamic range. For all of the ATP testing devices, the lower limit of quantitation will also require improved reliability towards and at the indicated “0” (zero) level. The most important cleanliness related

applications are for data produced at the lower end of the dynamic range and so this is the most important part of any rescaling consideration.

j. A common scale would provide the ATP testing devices with a quantitative basis on which standardised and uniform calibration could be achieved.

k. The ability to calibrate ATP testing devices would introduce the capacity to indicate accuracy of ATP measurements against a common scale to improve reliability. This common scaling would be the best platform on which to provide a competitive advantage between the commercial organisations, through improvements in both accuracy and precision in readings of ATP.

l. Once the accuracy of response to a calibrated range is established, variability would then be subject to competitive amongst manufacturers. The most competitive ATP testing devices would then have both measureable accuracy and calibration, with the added advantage of lower variability (better precision), whether due to improved reagent stability, light detection, or with better pricing.

m. The attainment of the goals outlined above can only be achieved through a properly constructed and validated measurement scale for ATP which is calibrated against a known quantity of ATP.

Researching a new method for cleaning product validation

The results shown in this PhD demonstrate that ATP testing devices reliably distinguish between ATP based data sets when tested on the basis of a ten-fold difference in underlying ATP. It is accepted that sanitising and cleaning compounds are intended to clean and remove up to 3 logs of bacterial soils.[6] The difficulty of using live bacterial suspensions for this testing includes both the cost and the time taken to achieve feedback (it is very time inefficient). A testing system that can provide measurements that indicate results in a quick and efficient manner would be a major benefit to both industry and healthcare settings where these cleaning products are used in this global market.

19. Researching a new test method cleaning product validation using ATP testing would include the following considerations:

a. There are additional methodological issues to be considered with any cleaning process such as wiping technique, wiping pressure, and wiping material characteristics.[7] However, the significant advantage of using a standardised ATP

testing approach would be the improved efficiency through reduced response time which would allow each of the variable to be controlled and independently measured, and have almost immediate feedback from the testing;

b. The faster feedback from ATP testing as opposed to standard microbiological techniques would provide the opportunity to increase the number of test replicates thus improving the reliability of any results;
c. The issue of relative scaling with RLU is not a problem as it has already been established that for each of the brands tested, the three log change in ATP is measured at the same response rate. Therefore, this work could be conducted irrespective of the brand and scaling approach used by the ATP testing devices;
d. The key qualifying feature of a three log diminishment in soiling could easily be validated against any of the ATP testing devices once the methodological approach to cleaning processes are considered.
e. The use of a "scrub testing" device similar to those used for paint and other surface coating testing systems would standardise many of the concerns such as wipe direction, wipe pressure and overall wipe repeatability.
f. The ATP enriched soiling systems would also then be validated using both ATP testing devices and compared with results using live bacterial suspensions in a controlled laboratory environment. In this way both the soils and the methods could be validated to be applied as a single test methodology;
g. The possibility of cleaning products affecting the ATP reaction and thereby causing interference in the RLU readings will also need to be broadly addressed. This would involve a number of fairly simple controlled experiments which as yet the literature has not reported. The findings using actual products would have immediate relevance to field usage of ATP testing devices and would be ideally published quickly as a short report or similar.

Researching a new sampling algorithm for ATP testing devices

20. The researching of a new algorithm approach, to guide the sampling methodology in the normal use of ATP testing could be used to define cleanliness thresholds based on a statistical model that could be applied to any brand of ATP testing device. This might be achieved even with the current and inadequate RLU scale.

Within healthcare settings there is a public health need to both characterise and standardise surface or environmental cleanliness. Without a common scale on which to measure ATP standardisation is probably not possible without a more complex and mathematically driven approach to both sampling and data interpretation.

So then, applying the principle established in the fourth published paper, i.e. that anyone reading has a 20% chance of being wrong by a factor of two, a bandwidth approach to data interpretation might be workable. This would allow any individual reading, or set of replicate readings, to be interpreted within a surrounding zone. This could also allow a cleanliness threshold to be determined that accounted for the current level of imprecision.

Further work on investigating this approach would be a useful and very practical application of the findings that could produce a useful platform for benchmarking studies within any healthcare settings. This approach might also be validated for cleanliness studies investigating cleanliness outcomes from medical device cleaning processes.

Applications in other fields such as environmental health could also be considered. One advantage of the environmental health applications is the criteria used for cleanliness monitoring. Cleaning in food preparation areas is measured on a clean versus unclean approach where a numerical cut-off or cleaning threshold is less important and undefined.

Extending ATP testing into Applied Epidemiology with Cleanliness and Cleaning Monitoring

21. The ultimate goal of cleanliness monitoring and cleaning monitoring is to improve the standards of hygiene within healthcare settings and to thus reduce the risks associated with MRO on uncleaned or insufficiently clean surfaces. There are an enormous range of potential confounding factors for a study of this type. In fact, there may need to be a layered approach of several studies, each of which isolates a limited number of factors, but each one builds on the results of the earlier findings.

The range of confounding factors includes all of the ATP testing issues identified in this thesis, and also factors such as cleaning materials, cleaning methods, cleaning frequencies, cleaning supervision, cleaning and cleanliness monitoring methods, management systems, quality assurance factors, hand hygiene compliance monitoring (ideally validated), patient monitoring and even cohorting, interlocutory microbiological testing of patients and environmental surfaces, microbial identification technologies including genetics (whole genome sequencing) to confirm identification of MRO and transmission associated events, initial epidemiology and follow up, properly designed and randomised trials and of course a cost versus benefit aspect on the processes.

Cost versus benefit is a critical feature of the infection prevention paradigm. Clearly the hazards presented by an insufficiently clean healthcare environment involve patient morbidity and potential mortality. However, the budgets within healthcare are not unlimited and the value of a life concept is to be compared against hard costs such as effective and efficient cleaning services, materials, and the management overheads required to ensure cleaning and cleanliness standards are set and achieved. These costs are also to be compared with the costs associated with a patient who sustains an HAI and then has extended treatments, length of hospital stay, and potentially diminished quality of life following the HAI.

These critically important matters will need to be addressed, but at this time the nature of the cost benefit study, and the epidemiology are beyond the boundaries for this thesis.

Continuing to promote and publish results from this PhD study

22. Publishing the results of this PhD more widely would also be useful in combating the "weight of numbers" approaches taken by ATP testing device promotors and manufacturers.[8] There has been a number of abstract submissions for conferences in 2016 that are being submitted in the normal manner.

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Section Two

The five publications

The published papers appear in the chronological order of publication

1st paper

Journal:	**Healthcare Infection (HI): 2012:17:91-97**
Authors:	**Whiteley GS, Derry C, Glasbey T.**
Title:	**The comparative performance of three brands of portable ATP-bioluminometer intended for use in hospital infection control**

2nd paper

Journal:	**Infection Control & Hospital Epidemiology (ICHE): 2013:34:5:338-540**
Authors:	**Whiteley GS, Derry C, Glasbey T**
Title:	**Reliability testing for portable adenosine triphosphate bioluminometers**

3rd paper

Journal:	**American Journal of Infection Control (AJIC): 2015:43:147-153**
Authors:	**Whiteley GS, Derry C, Glasbey T**
Title:	**Failure analysis in the identification of synergies between cleaning monitoring methods**

4th paper

Journal:	**Infection Control & Hospital Epidemiology (ICHE): 2015:36:6:658-663**
Authors:	**Whiteley GS, Derry C, Glasbey T, Fahey P**
Title:	**The perennial problem of variability in adenosine triphosphate (ATP) tests for hygiene monitoring within healthcare settings**

5th paper

Journal:	**American Journal of Infection Control (AJIC): 2015:43:1270-1275**
Authors:	**Whiteley GS, Knight JL, Derry CW, Jensen SO, Vickery K, Gosbell IB**
Title:	**A pilot study into locating the bad bugs in a busy intensive care unit**

Chapter 5

The comparative performance of three brands of portable ATP-bioluminometer intended for use in hospital infection control

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The comparative performance of three brands of portable ATP-bioluminometer intended for use in hospital infection control

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Abstract. ***Background*:** Portable adenosine triphosphate (ATP)-bioluminometers have been used in the food industry to monitor the effectiveness of surface cleaning but their intended use in hospital infection control suggests a need for instrument validation to confirm effective technology transfer.

***Methods*:** The performance of three readily available brands of portable bioluminometer was compared in terms of their ability to generate relative light units (RLU) from a range of standard ATP solutions. Quality control of standards was carried out using high pressure liquid chromatography (HPLC).

***Results*:** There was no significant difference ($P = 0.05$) in the ability of different meters to effectively measure hygiene change on a log scale in a central measurement range of 0.001 to 1.0 mg L^{-1} ATP. Outside this range meter performance deteriorated, with the possibility of individual and comparative measurement error. No out-of-range warning system existed for any of the meters. While different brands generated widely different $\log_{10}$RLU values for fixed quantities of ATP in this range, curve similarities suggested standardisation possibilities to enable comparison of results. Testing at a higher level of resolution in the 0.0001 to 0.002 mg L^{-1} ATP range proximate to a proposed 100 RLU cleaning benchmark also revealed poor repeatability as a potential for measurement error.

***Conclusions*:** Portable ATP-bioluminometers, when used to indicate surface cleaning effectiveness, demonstrate reliable performance when measuring over a very wide range of ATP concentrations. Monitoring hygiene in terms of an absolute threshold value such as a cleaning benchmark may, however, be invalid as a concept when using existing portable ATP-bioluminometer technology.

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Introduction

Assessment of the effectiveness of hospital surface cleaning in Australia relies primarily on direct visual examination, a practice enshrined in National infection control guidelines.[1] Although unreliable, visual assessment persists because of simplicity of approach and immediacy of feedback to guide the cleaning process.[2,3] The alternative of microbiological monitoring offers the advantage of quantitative results for changes in surface loads of indicator organisms and pathogens, but demands time and technical expertise which are not always available. To address these issues several innovative methods of cleaning assessment combining accuracy, quantification and immediacy of results are under consideration internationally.[4]

One possibility is the use of fluorescent 'soiling spots' marked on high touch objects (HTOs) or near-patient surfaces before cleaning, with subsequent examination under ultraviolet light to detect cleaning efficacy through either full or partial removal.[5,6] While the chemiluminescent technology is simple and immediate, results do not measure soiling but rather cleaning efficiency and readings are qualitative only. Another approach relies on portable bioluminometry to measure adenosine triphosphate (ATP) residues on relevant surfaces, potentially yielding quantitative results.[7] While this has been successfully used in the food industry for at least 15 years to monitor contact-surface hygiene, validation for use in the hospital setting still requires attention, and this served as motivation for the research described in this paper.

ATP is the energy currency of living cells and is, therefore, capable of acting as proxy for a broad range of contamination types in the hospital setting, including microorganisms, human cells, food waste, vomitus and faeces.[8,9] ATP-

Implications

- Portable ATP bioluminometers reliably indicate largely different ATP levels.
- Monitoring of hygiene using an absolute threshold such as a cleaning benchmark may be invalid using existing technology.
- The paper suggests that equipment design and ATP measurement scaling be revised before the equipment is accepted for use within healthcare settings.

bioluminometry is not, however, specific for these substances or for any microbial indicator or pathogenic species.[10] ATP detection alone cannot indicate factors such as infectivity, virulence or infectious dose that are related to disease transmission risk, which cannot be assessed without identification of specific microorganisms.[11] ATP monitoring can, however, act as a proxy for surface hygiene change associated with adequacy of cleaning practice.[12]

The ability of commercially available portable bioluminometers to achieve accurate ATP measurement depends on several factors, the most basic of which is the technical performance of the instrument itself.[13] It is, therefore, surprising that minimal research attention has been given towards instrument validation before the conducting of intervention research studies in the hospital setting.

In validating any instrumentation the intended operational environment must be taken into account. Important differences may exist between the hospital sector, where the use is intended, and the food industry where the meters have been successfully used in the past. Preliminary investigation showed that in the food industry the instruments are primarily used in hazard analysis critical control point (HACCP) assessment, aimed at monitoring well defined control points in relatively linear food production processes, with risk parameters lying within circumscribed ranges.[14–16]

Hospital monitoring presents fundamental differences, including the very wide range of environments and surfaces to be monitored, the *ad hoc* nature of the cleaning process required at any time, and the unpredictability of contaminant level and type to which a surface may be exposed.[17] In addition, an interest in comparing results for different hospitals and units, where different brands of meters with different performance characteristics may be in use, has been identified.[18]

In terms of the different operating principles employed by different instrument manufacturers, it is important not only to validate ATP-bioluminometry in general for hospital use, but to include different brands of instrument in such validation.[19] The authors see the ultimate establishment of validation criteria for portable bioluminometers as a complex process requiring clear definition of healthcare sector objectives and determination of the capacity of the main types of unit to meet these objectives. The paper discusses findings on the reproducibility of ATP readings relating to individual and comparative metering performance of three existing brands with the aim of encouraging dialogue on the validation issues identified.

The fundamental principle of all ATP-bioluminometry is the measurement of light from the two-stage luciferin reaction, which give fireflies and glow-worms their 'glow':

(i) luciferin (in reagent) + ATP (from surface) → luciferyl adenylate + inorganic pyrophosphate

(ii) luciferyl adenylate + O_2 → oxyluciferin + AMP + visible light (read by bioluminometer)

While this biochemical reaction is highly efficient in terms of chemical to light energy transfer, it presents challenges to those wishing to capture the reaction chain *in vitro*, with manufacturers using a variety of reagents and metering arrangements to achieve their aims.[20] In anticipation of variations in results during different monitoring events, bioluminometer read-out is given in relative light units (RLU), based on ordinal rather than interval scaling. Ordinal scales allow results in a specific series of observations to be ranked against one another, although numerical differences between successive values may not be constant, in contrast to those in interval scales such as the Celsius temperature scale. Unfortunately there is no benchmark to determine what constitutes a 'specific series of observations' in bioluminometry so that monitoring at different times, in different settings, and with different brands or designs of meter might produce unwanted variability in the results.

While attempts have been made in the past to test meter performance in terms of microbiological standards relating to pathogens and indicator counts, several complications have intervened, including variation in ATP level based on different species and phase of growth.[21–23] This potential confounder was avoided in the current project by assessing RLU generated against standard ATP solutions monitored for stability at regular intervals using calibrated HPLC.

Materials and methods

Three commercially available portable ATP-bioluminometer kits and fresh manufacturers' reagent tubes including swabs were obtained from laboratory suppliers in the Sydney region. Two of the brands were from large multinational manufacturers and had been referred to by name in healthcare publications in the United Kingdom and United States of America, whereas the third was a relatively new brand. Other brands are believed to exist. In this paper the brands are described as Brands A, B and C for purposes of anonymity. Manufacturers' packaging claims suggested suitability of the bioluminometers for use in both food and healthcare settings.

ATP was obtained from Sigma Aldrich (Castle Hill, NSW, Australia) and a nine-step, 10-fold dilution series in the 0.00001 mg L^{-1} to 1000 mg L^{-1} range was made up using a phosphate buffer diluent immediately before validation testing. An additional five-step, linear dilution sub-series was made up in the 0.0001 to 0.002 mg L^{-1} ATP range to assess

meter resolution within a critical ATP band relating to a recently proposed 100 RLU hospital cleaning benchmark.[24] ATP solutions were stored in sterile equipment, on ice during each bioluminometer assessment run.

The bioluminometers were set up as per the manufacturers' instructions and the reagent tubes checked for freshness in terms of expiry date. In using the bioluminometers the manufacturers' instructions were adhered to, which typically involved placing the manufacturers' swabs with collected ATP in the tubes provided, releasing the reagent, mixing for a controlled period, and positioning the tube in the bioluminometer for reading. To remove potential errors relating to surface swabbing, however, the recommended swabbing procedure was modified by directly applying a 20 µL aliquot of each solution in the standard ATP series onto each swab using a 10–100 µL calibrated micropipette, with new micropipette tips for each standard. The amount of 20 µL was selected by empirically determining the absorptive capacity of each manufacturer's swab type, and using the lowest capacity which could safely be absorbed without risk of over-saturation. Assessment was repeated six times for each bioluminometer at each ATP concentration, and where more than two swabs exceeded the interquartile range for measured ATP, five additional swabs were tested at that concentration. This yielded a total of 95, 93 and 90 readings for bioluminometers A, B and C, respectively.

Quality control for ATP solutions was carried out using high pressure liquid chromatography (HPLC) before and after each test series. The standard curve for the stock solution was derived from a certified ATP standard (Sigma Aldrich), calibration samples being run in triplicate, with results showing strong positive linear correlation ($r^2 = 0.9998$).

Raw RLU data generated at each ATP concentration was saved in Excel spreadsheets (Microsoft, Seattle, WA, USA) and median, interquartile and total ranges estimated. Non-parametry of data was confirmed using a Normal Probability Plot and a relevant test of statistical significance (Kruskal–Wallis) applied using Minitab (Minitab Inc., State College, PA, USA) to identify association between related datasets at the assumed level of significance ($P = 0.05$). Graphs of overall performance, and performance at the hypothesised cleaning benchmark were prepared using PASW (IBM, New York, NY, USA).

Results

Curves were generated for the bioluminometers using logarithmic scales for both RLU and ATP to accommodate the very wide metering range and to assist visual interpretation of results (Fig. 1). Median values for RLU at each ATP concentration were used as a summary index in preference to means, in terms of the relatively small datasets with high interquartile and total ranges.[25]

A test of association indicated no significant difference between performance of the three bioluminometers in the middle of the measurement range, from 0.001 to 1 mg ATP L^{-1} ($P < 0.39$), when logs of both RLUs and ATP median values were analysed. The Chi-square test, however, assesses ratio relationships and marked differences in absolute values of RLU at specific ATP concentrations existed (curves are

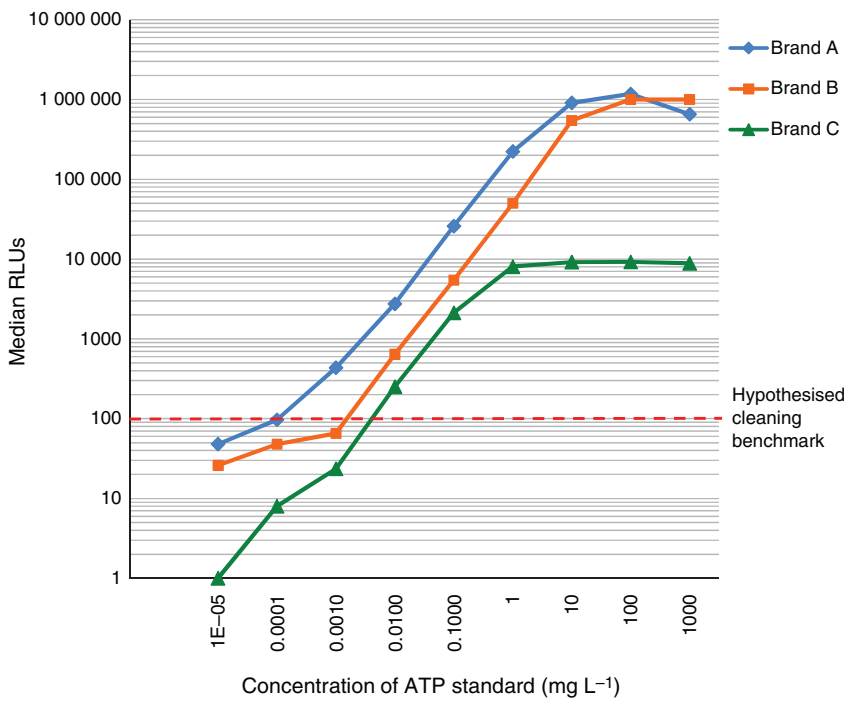

Fig. 1. Performance curves for three brands of portable ATP-bioluminometer.

parallel but not coterminous). For example, at the 0.01 mg ATP L^{-1} standard there is an 11-fold difference in RLU between the most and least optimistic instruments.

In terms of the upper end of the range (1 to 1000 mg ATP L^{-1}), a significant difference in performance was found to exist between the bioluminometers ($P < 0.03$), as suggested by the convergence of curves for two of the meters, with rapid flattening and divergence of the curve for the third meter. This may relate to differences in light measuring technology between the meters, including variations in the chemical extinction point for the available luciferin reagent.

At the lower end of the range (0.00001 to 0.001 mg ATP L^{-1}) there was also a fall-off in monitoring performance, with significant differences between the three brands ($P < 0.04$). None of the meters, however, had a warning system or automatic shut down to alert the user to the fact that it was operating outside its apparent effective (accurate) range.

Next the ability of meters to measure fine changes in ATP level in the vicinity of an absolute cleaning benchmark, as recently proposed in the literature, was assessed.[23] This cleaning benchmark is basically an action-threshold limit value (TLV) which is intended to indicate when a sufficient state of hygiene exists for the surface to be considered 'safe', and does not require further cleaning.

Five linear sub-dilutions of standard ATP, lying within the range 0.0001 to 0.002 mg L^{-1}, which relates to the potential 'window' in which the cleaning benchmark subsists, were tested. The results are presented in Figs 2 to 4 as line graphs with box and whisker plots indicating spread of data at individual ATP concentrations. It should be noted that ATP levels in these graphs are in µg L^{-1} and that end points of the curves do not comply exactly with those shown in Fig. 1 because different median values are in use. Proximity of the line graphs to the 100 RLU cleaning benchmark does not indicate meter accuracy but is an artefact based on window selection.

A test of association showed significant difference in monitoring ability for median RLU generated by the three brands at this level of resolution ($P < 0.002$). While the graphs suggested that each meter was capable of generating a relatively linear response based on median data from four to five swabs for each ATP value, interquartile and total ranges for RLU at specific concentrations of ATP was unacceptably large, in many cases overlapping the range of adjacent measurements.

While the preliminary validation described here essentially involved the capacity of the instrumentation to accurately

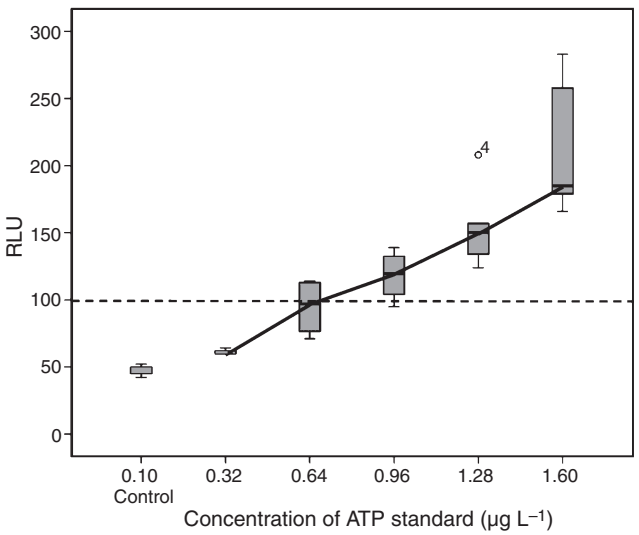

Fig. 3. Performance curve for Bioluminometer B, proximate to the 100 RLU cleaning benchmark.

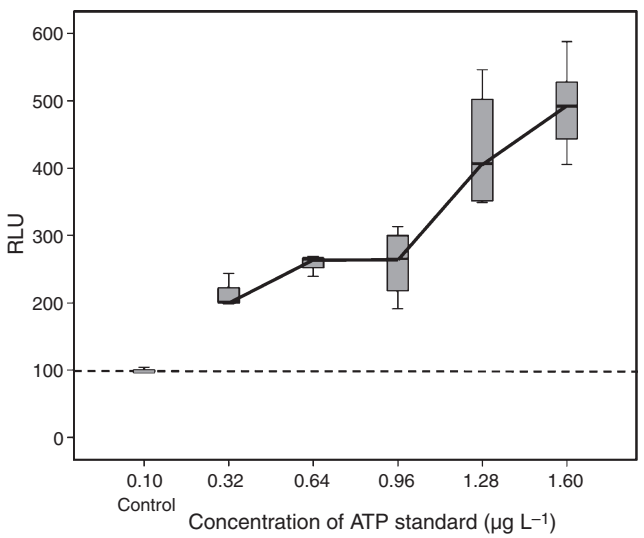

Fig. 2. Performance curve for Bioluminometer A, proximate to the 100 RLU cleaning benchmark.

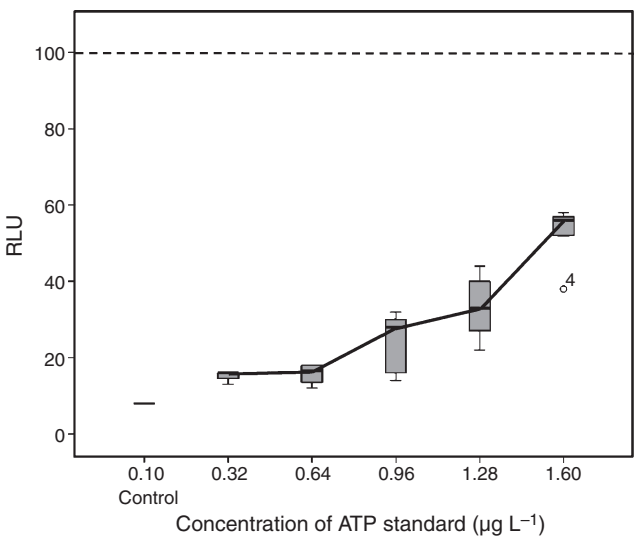

Fig. 4. Performance curve for Bioluminometer C, proximate to the 100 RLU cleaning benchmark.

measure ATP concentration, it was noted that differences which might impact on ultimate monitoring accuracy existed in ancillary equipment. For example, different brands of swabs had different absorptive capacities for fluid, which might influence uptake and release of ATP.[26,27] The impact of these differences on meter validation still needs to be explored.

Discussion

Environmental hygiene within hospitals has a direct impact on rates of cross infection with key microorganisms and effective cleaning has been shown to reduce rates of infection.[28,29] To assess the effectiveness of cleaning it is important to scientifically verify the cleaning processes to ensure that improved hygiene has been achieved and that the surface is sufficiently clean to reduce the risk of cross infection.[30,31] It has been proposed that portable ATP bioluminometry can be used as a reliable method of verifying cleanliness standards on environmental surfaces and objects in place of routine microbiology. Work in Australia using ATP as an indicator of surface hygiene has examined applications including medical device cleaning and more broad applications in environmental surface cleanliness.[32–34]

The results of our study suggest that portable ATP-bioluminometers have capacity to generate data over a wide effective measurement range, which augurs well for cleaning assessment in the hospital setting where surface soiling can vary from the invisible to the excessive. Performance characteristics, however, vary with brand and this needs to be addressed if error is to be avoided during comparative assessments with different meters.[21]

The three meters produced very similar response to surface hygiene change in terms of $\log_{10}$RLU within an effective measurement range, as shown by the close similarity of parallel traces in the central portion of the graph in Fig. 1. While the traces are currently displaced in relationship to one another, synchronisation could be achieved by simple manipulation of the *y*-axis intercept value, c, in the straight line formula $y = mx + c$. This suggests potential for standardisation of $\log_{10}$RLU results for the different brands, facilitating comparative assessments.

In practice, this could be achieved using one of two approaches. The first would involve the determination of an industry standard for $\log_{10}$RLU generated by ATP standards within an accepted effective metering range. Meters would then need to be adjustable (or have an internal data adjustment capacity) to standardise the ATP values currently generated to this range. Alternately, manufacturers could produce $\log_{10}$RLU curves for existing meters, similar to the ones shown in Fig. 1, enabling the user to carry out standardisation, or inter-meter comparisons, themselves. Given that the hospital industry is frequently time poor, metered adjustment might be preferred.

Having established that hygiene change can easily be interpreted when values are available as $\log_{10}$RLU, it is logical to suggest that portable bioluminometers should display results in log units as well as the current numerical units. This would also facilitate their meaningful use over a very wide range of surface contamination levels. Logarithmic interpretation of data has long been applied in bacteriology where a sliding scale of measurement sensitivity has been needed to cope with exponential growth of microorganisms. Ready availability of $\log_{10}$RLU data in bioluminometry might well lead to synergies between bioluminometric and microbiological monitoring in hospitals.

An important validation criterion for any meter should be its ability to warn the user or shut down when it is operating outside its effective measurement range. Figure 1 shows that at the upper end of the effective measurement range for each meter there is a point at which results cease to be comparable, with a rapid flattening of response in all meters. Individual measurements taken within this range run the risk of generating lowered results, which may potentially reduce cleaning imperative. At the lower end of the range there is also a levelling out in meter response, with potential to produce misleading results in the vicinity of the proposed cleaning benchmark. Earlier work using quantitated live microbes indicated that reliability of the ATP bioluminometers is questionable at the lower end of readings.[19]

A broad discussion on the concept of a ‘cleaning benchmark’ is needed. Certain researchers have proposed this type of action-threshold limit value (TLV) for RLU as a point below which contamination is so low that the surface can be assumed clean and unlikely to act as a source of infection. To determine this point, measured ATP values may have to be related to microorganism presence, and critics have pointed out that there is a highly inconsistent relationship between ATP and number of microorganisms present based on interspecies variations, growth phase and a range of other variables.[10,11]

Originally proponents of an absolute TLV proposed 500 RLU,[7,3] which was subsequently reduced to 250 RLU,[23] and finally 100 RLU.[24] The results from this validation study indicate that the currently proposed 100 RLU level is disturbingly close to the lowest range of effective measurement capacity of all three meters (Fig. 1).

In order to assess the ability of the three brands to accurately measure ATP at a high level of resolution proximate to the 100 RLU cleaning benchmark, the study within the 0.0001 to 0.002 mg L^{-1} ATP range was carried out (Figs 2, 3 and 4). A potentially unacceptable level of variance for each of the meters was found to exist, with values in interquartile and total range for RLU tending to overlap for adjacent values in the linear series. It would seem, therefore, that fundamental problems with the concept of a cleaning benchmark and the apparent inability of the existing technology to accurately measure at this point give rise to validation problems relating to this intended use.

While the study is based on limited data for three instrument brands, it is hoped that it will encourage discussion between the healthcare sector and the bioluminometer industry, in order that the prerequisite of developing detailed

validation criteria and manufacturer specifications can be attended to as precursory to investigative studies based on ward monitoring. To achieve this, further research is required into the extent and intended use of the instruments, their measuring characteristics and capacity, the format and quality of data required for integration into ward-focussed assessment, and the nature of the integrative risk monitoring framework to be informed in the future by hospital bioluminometry.

Conflict of interest

Greg Whiteley is a PhD candidate at the University of Western Sydney (UWS) and with Trevor Glasbey is an employee of Whiteley Corporation. Chris Derry is principal lecturer and research supervisor in environmental epidemiology and toxicology in the School of Science and Health, UWS. None of the parties has any commercial association or can gain benefit from any bioluminometer manufacturer or supplier.

Funding sources

Consumables were funded by the UWS PhD support program and by Whiteley Corporation. Devices were provided by UWS and Whiteley Corporation. No ATP meter brand provided authorisation or fiscal support for the study and there is no connection between Whiteley Corporation and any agency or company which materials were used in this study.

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Chapter 6

Reliability testing for portable Adenosine Triphosphate bioluminometers

RESEARCH BRIEF

Reliability Testing for Portable Adenosine Triphosphate Bioluminometers

Measurement of adenosine triphosphate (ATP) using portable bioluminometers has been adapted from the food manufacturing sector, and it has been suggested that it could be used as an indicator of surface soiling or cleanliness in hospital settings.[1] Some healthcare authorities are considering the use of portable ATP bioluminometers as a tool for standard setting for surface cleanliness to improve cleaning standards.[2] Central to this approach is the use of a commonly accepted level of detected ATP—expressed as relative light units (RLUs)—that may be used as a surrogate for underlying soiling, including the presence of pathogenic microorganisms.[3]

It has been demonstrated that improvements can be made to cleaning processes with fluorescent markers through a simpler approach that provides a qualitative efficiency measurement of the cleaning process.[4] Measurement of surface hygiene using ATP bioluminometers is thought to provide a more quantitative surrogate of surface cleanliness.[5]

A proposed ATP/RLU standard for acceptable cleanliness has been revised from 500 RLUs, originally suggested by Griffith et al,[1] to 250 RLUs by Lewis et al[6] and most recently to 100 RLUs by Mulvey et al.[7] However, a close examination of the references reveals that different brands of ATP bioluminometers were used to establish the data in each reference—a substantial problem given that each unit reads on a different relative scale.[8] Even with a single branded unit variable results are observed without a clear explanation.[9] There also is debate over the validity of using ATP measurement as an analogue for surface soils and the presence of pathogenic microorganisms.[10]

Our aim was to validate the reliability of measurement of 3 commonly available brands of portable ATP bioluminometers. Two of the brands selected (Hygiena and 3M) feature prominently in the published literature, and the third brand (Kikkoman) provided a different approach to luciferase presentation (a powder rather than a preprepared liquid).

Our method was selected to minimize confounding variables, such as brand-to-brand differences in RLU scaling, swab absorption, cell lysis mechanism and efficiency, liberation of cellular ATP, and variations in cellular ATP during bacterial cycles. To achieve this, the method used an ATP source of known purity (Sigma-Aldrich). The ATP was diluted across multiple dilution series, which enabled testing of the 3 devices across the full dynamic range of detection for each device, from the lower limit of detection to response tapering. We included multiple 10-fold dilution series as well as multiple narrower-range dilution series. A calibrated micropipette (Thermo Scientific) was used to apply the diluted ATP directly onto the swabs for each of the portable ATP bioluminometers, following an earlier method.[11]

At each dilution point, each brand was tested in triplicate or more frequently. The swabs for each brand were from multiple batches, stored in accordance with the manufacturers' recommendations, and used within the use-by dates. High-performance liquid chromatography (HPLC; Shimadzu) was used to validate accuracy, precision, specificity, and linearity and as a quantitative control for ATP.

Materials used in our experiments included 667 ATP swabs in 153 separately measured dilution series (3M: 246 swabs in 57 runs; Kikkoman: 222 swabs in 49 runs; Hygiena: 199 swabs in 47 runs). HPLC data were generated over 22 runs against 72 dilutions. ATP concentrations ranged from 10^{-5} mg/L to 1,000 mg/L.

Similar to many other studies, our results indicated considerable variability; data are shown in Figure 1. The raw data were subjected to test of association, which found no significant difference. The nonstandardized RLU scaling, which was different for each brand, required a single common calculation for comparison. The coefficient of variance was determined to be the best method of expression of the variability in RLU readings. The experimental method of multiple runs (153 test runs plotted in Figure 1) over the full dynamic range of detection did not allow for an overall standard deviation or mean to be determined for each device. HPLC demonstrated a lack of variability with excellent precision and repeatability of ATP analysis, provided that the limits of detection were observed.

The variability of the coefficient of variance shown in the ATP data for the 3 brands strongly suggests that these analytical systems were rarely able to produce a reliable response no matter the dilution or quantity of ATP. These findings have implications for interpretation of data obtained using a portable ATP bioluminometer when monitoring surface hygiene as part of infection control practice. Use of these devices in the food sector is based on a validation pathway quite distinct from the way in which usage has been trialed in the healthcare setting.[1,3]

Portable ATP bioluminometer units are reliable for distinguishing between very different levels of ATP where the concentration is varied by at least an order of magnitude, thus reducing the relative impact of the variance.[8] The usefulness of these units in healthcare settings is not ruled out by our findings provided that usage allows for wide disparity in narrowly focused measurements of similarly clean or soiled surfaces. We caution against use of a finite measure of cleanliness based on RLUs until standard measures are improved and the units are able to read reliably on a common RLU scale, with a clear association with quantitated ATP levels.

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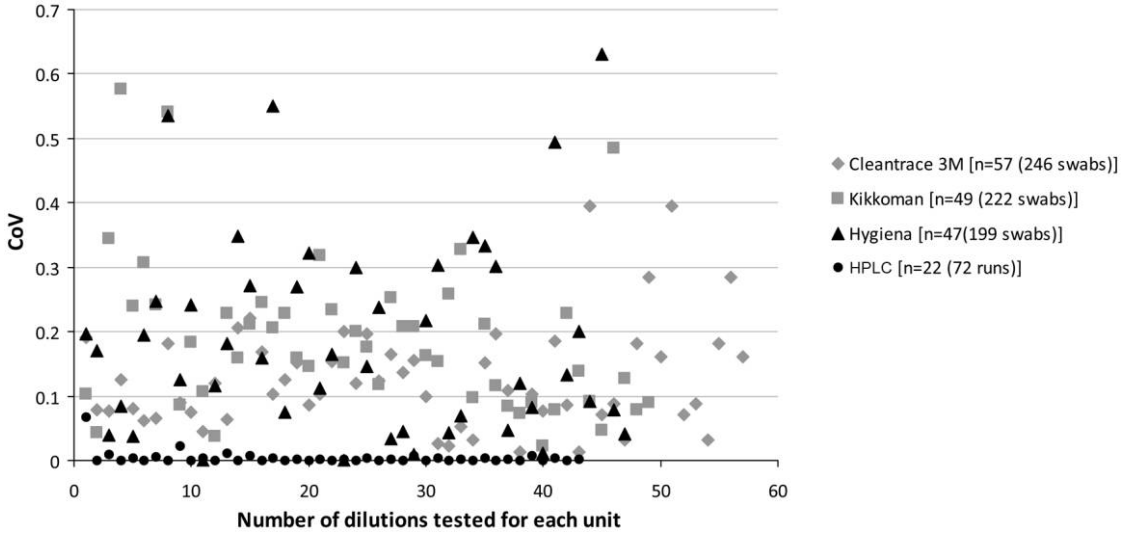

FIGURE 1. Coefficient of variance (CoV) for 3 portable adenosine triphosphate bioluminometers. *n* values indicate the number of separate dilutions tested per brand. HPLC, high-performance liquid chromatography.

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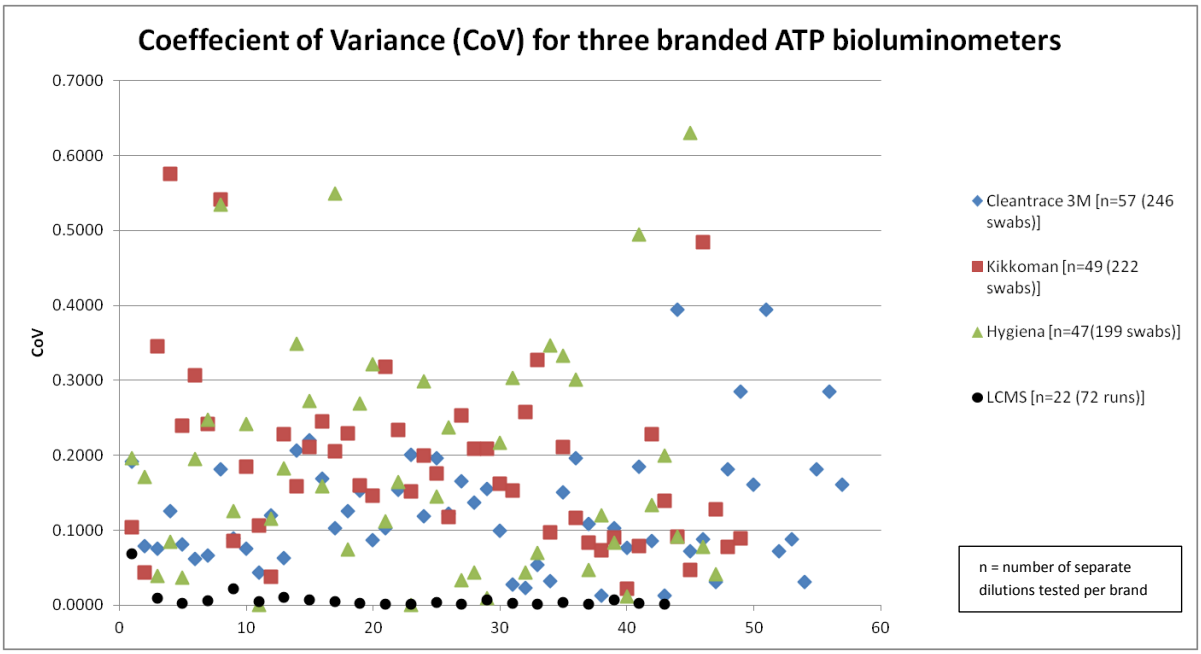
Coeffecient of Variance (CoV) for three branded ATP bioluminometers
CoV
0.7000
0.6000
0.5000
0.4000
0.3000
0.2000
0.1000
0.0000
0
10
20
30
40
50
60
Cleantrace 3M [n=57 (246 swabs)]
Kikkoman [n=49 (222 swabs)]
Hygiena [n=47(199 swabs)]
LCMS [n=22 (72 runs)]
n = number of separate dilutions tested per brand

Chapter 7

Failure analysis in the identification of synergies between cleaning monitoring methods

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Major article

Failure analysis in the identification of synergies between cleaning monitoring methods

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Key Words:
FMEA
ATP
FM
Visual inspection
Microbial recovery

Background: The 4 monitoring methods used to manage the quality assurance of cleaning outcomes within health care settings are visual inspection, microbial recovery, fluorescent marker assessment, and rapid ATP bioluminometry. These methods each generate different types of information, presenting a challenge to the successful integration of monitoring results. A systematic approach to safety and quality control can be used to interrogate the known qualities of cleaning monitoring methods and provide a prospective management tool for infection control professionals. We investigated the use of failure mode and effects analysis (FMEA) for measuring failure risk arising through each cleaning monitoring method.
Methods: FMEA uses existing data in a structured risk assessment tool that identifies weaknesses in products or processes. Our FMEA approach used the literature and a small experienced team to construct a series of analyses to investigate the cleaning monitoring methods in a way that minimized identified failure risks.
Results: FMEA applied to each of the cleaning monitoring methods revealed failure modes for each. The combined use of cleaning monitoring methods in sequence is preferable to their use in isolation.
Conclusions: When these 4 cleaning monitoring methods are used in combination in a logical sequence, the failure modes noted for any 1 can be complemented by the strengths of the alternatives, thereby circumventing the risk of failure of any individual cleaning monitoring method.

Cleaning in health care settings is a manageable activity that can be audited for consistency and quality.[1] The processes of cleaning environment surfaces within hospitals can be monitored by 4 distinct methods: visual inspection, microbial recovery, rapid ATP bioluminometry detection, and use of fluorescent marker (FM) technologies.[2] Each of these monitoring methods generates a distinct type of information that is difficult to integrate into a single monitoring result.[3,4]

Although cleaning has the goal of removing soils and pathogens, the monitoring methods used for management and supervision of cleaning have a distinct quality assurance role.[5] The goal of any monitoring method is to provide feedback on cleaning failure to assist in the management and improvement of environment cleaning within health care settings.[6-9]

The sampling approach taken for the monitoring of environment surfaces within health care settings is a constant problem.[10,11] The apparently random distribution of soils, including dry surface biofilms and pathogens, presents a challenge for any sampling plan investigating the nature of environment contamination, and is complicated by the presence high-touch objects.[12-14] Health care environment cleaning protocols are management tools that set out the practical steps to achieving the goal of removing soils and improving the quality of environment surface hygiene.[15,16]

Health care cleaning processes are designed on a risk-based format with the highest risk areas requiring the most frequent or highest-intensity cleaning.[17] The secondary process of cleaning monitoring is intended to ensure that soil removal goals are met with optimal efficiency and efficacy.[18]

If the cleaning monitoring method is flawed due to uncontrolled or unrecognized failure, then the data on cleaning outcomes will also be flawed and unreliable. This compromises the management goal of ensuring that the primary process of cleaning has been achieved.

We focused on the failure of cleaning monitoring methods and not on the actual processes of cleaning. Failure mode and effects analysis (FMEA) is a reliable safety and quality management risk-assessment tool that identifies potential failure conditions or errors that may cause failure for products or processes.[19] The FMEA

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applied here investigated if current cleaning monitoring methods could be optimized to reduce cleaning failures as a type of medical error.[20,21]

Within this management context, FMEA as a risk tool can add value by systematically examining failures to mitigate or minimize their influence on the cleaning process being undertaken.[22] An ultimate aim is the identification of failure modes within cleaning monitoring systems as a quality improvement process in health service provision.[23]

FMEA is typically conducted on products or processes, through the application of existing information on identified failures or failure modes to anticipate failure events. A failure mode is defined as a "loss of intended function" under normal operating conditions. FMEA was selected as a suitable method for prospective risk assessment of the 4 monitoring methods due to its applicability as a forecast model and as a risk-assessment tool that is frequently used in the context of medical devices.[24]

A risk-assessment team of 3 individuals with collective skills in FMEA and cleaning monitoring using each of the 4 methods was formed, with the following aims:

- To identify a modified approach for the application of FMEA in failure analysis relating to the 4 commonly used cleaning monitoring methods,
- To test the feasibility of the approach by carrying out a preliminary assessment of the 4 monitoring methods using the modified FMEA approach and to identify areas of commonality and work toward a monitoring model that could include the 4 methods in an integrated monitoring approach, and
- To identify strategies for the development of potential synergies during an integrated application of the 4 cleaning monitoring methods.

For each of the identified failure modes, literature support was required as part of the process of identification and consideration of mitigation for each major failure mode.

METHODS

The initial step in the FMEA process was for the FMEA team members to identify all possible causes of failure (loss of intended function) for each of the 4 cleaning monitoring methods.[24] Each identified failure mode was substantiated by relevant material in the literature. Where similar, multiple, or overlapping causes of failure were identified, these were gathered under a common failure mode. A comprehensive list of failure modes was noted for each cleaning monitoring method.

The risk associated with each of the failure modes was then assessed individually by each of the FMEA team members. Each of the failure modes was graded against 3 distinct categories with associated risk criteria. These categories were first graded for the likely frequency of occurrence of that failure mode during normal use, second for the severity of the effect of this failure mode on the validity of the information produced, and finally the assessment was also graded for whether the failure mode had any detectability in the normal course of its use. The grading system applied for this study is shown in Table 1. It uses a 3-tiered scoring approach similar to the 3-tiered risk criteria that are used in Australian Infection Prevention.[23] In the FMEA method used in our study, each category was assigned a score of 1-3 (low to high).

After the grading of each failure mode against each of the 3 categories (frequency, severity, and detectability), the grades for each failure mode were multiplied to produce a single score known as the risk priority number (RPN). The RPN is an overall indicator as to whether the failure mode requires further consideration or mitigation to minimize the identified failure risks. Whereas some authors have suggested use of weighting to accentuate critical failures, for the purposes of simplicity no weighting of the RPN was used in our study.[25] Using the 3-point grading system outlined in Table 1, there are only 10 possible RPN scores, with a minimum score of 1, a median of 7, and a maximum score of 27. The RPN for each failure mode was noted and ranked from high risk to low risk of the nominated failure occurrence.

Table 1
Ordinal conversion table for assigning values to identified failure risk cofactors

Failure risk cofactor and criteria	Descriptor	Value
Frequency		
The failure mode is unlikely to occur, or cannot occur, during normal monitoring operations	Low risk	1
The failure mode is fairly likely to occur during normal monitoring operations	Medium risk	2
The failure mode is highly lightly to occur, or always occurs, during normal monitoring operations	High risk	3
Severity		
Occurrence of the failure mode will have minimal or no effect on the monitoring results, or on the associated cleaning outcome	Low risk	1
Occurrence of the failure mode will have some effect on the monitoring results, or on the associated cleaning outcome	Medium risk	2
Occurrence of the failure mode will have considerable or extreme effect on the monitoring results, or on the associated cleaning outcome	High risk	3
Detectability		
Occurrence of the failure mode is easy to detect. Feedback is likely to inform immediate monitoring-failure mitigation	Low risk	1
Occurrence of the failure has a possibility of being detected. Feedback may inform early monitoring-failure mitigation	Medium risk	2
Occurrence of the failure mode is difficult or impossible to detect. Feedback is unlikely to inform monitoring-failure mitigation	High risk	3

Following the establishment of each RPN a structured dialogue was conducted to arrive at a common view on the RPN score using a modified Delphi approach.[26] The FMEA team members then reconfirmed each identified failure mode through the published literature and where no published evidence in support of the failure mode was available then that failure mode was discarded.

The RPN scores were finally ranked and divided into 3 classes based on a low, medium or high risk classification.[23] For ease of interpretation, the failure modes with RPN scores less than the median of 7 were accepted as low risk. Scores >7 and <13 were set as medium risk. Scores >13 were accepted as high risk.

The combined FMEA results were then considered to investigate whether combinations of the cleaning monitoring methods would provide mitigation of the effects of failure modes. This allowed for the FMEA team members to identify any novel approaches that could provide an enhanced approach to cleaning monitoring. This step allows for mitigation to be used as a form of redundancy whereby a reduction of the overall risks arising from cleaning failure is practically achieved by reducing the risks of failure of the cleaning monitoring methods. Thus the risk assessment approach is used as predictive tool to improve practice in advance of failure. This will lead to further research opportunities.

RESULTS

For the 4 cleaning monitoring methods 32 failure modes were identified and risk-assessed. Table 2 shows the 15 failure modes

ultimately found to be associated with the highest RPNs and associated failure risk.

The consideration of mitigation identified a novel combination of the 4 cleaning monitoring methods. This was particularly crucial for the high and medium risk failure modes. The new model for cleaning monitoring is outlined in Figure 1.

Costs were considered on a desktop basis for the purposes of cost consideration on both individual basis and as a combination of all 4 methods.

The high and medium risk failure modes identified using FMEA for each of the cleaning monitoring methods is noted below for each of the cleaning monitoring methods.

Visual inspection

The highest RPN was for visual inspection. Although a surface may look clean, human vision cannot detect microscopic-level contamination.[11] This method is the current standard approach for normal cleaning monitoring within Australian and American health care settings.[27,28]

Similarly, visual inspection cannot detect nonpathogenic soils that can be left in situ due to a flawed cleaning method, or a lack of cleaning.[6,7,12]

Visual triggers for cleanliness have a major behavioral effect and cannot be discounted as important.[29] The usefulness of visual inspection is that it is the only cleaning monitoring method that assesses the visual appearance of the hospital, which is also seen by patients, their families, and the other noncleaning staff members. Visual inspection is the only cleaning monitoring method that matches the appreciation of hospital cleanliness with all of the stakeholders and that provides a representative outcome of the hospital aesthetics. It is also noted that visual inspection carries only an existing cost overhead in terms of staff time used.

Mitigations noted for visual inspection included each of the other cleaning monitoring methods.

Microbial recovery (environmental Swabs)

A high RPN and high risk classification was also associated with the delay between microbial sampling and reading results, which frequently takes longer than 48 hours.[27,28] This delay renders cleaning monitoring results to low relevance because the patient, the cleaning processes, and the environment may have changed several times in the interim.[10]

Medium and high risk was associated with technical swabbing issues and poor sampling planning.[11] Sampling planning is particularly vulnerable to statistical error in terms of representative sampling due to the low level of sampling and the high surface area for potential contamination, even with high-touch objects or surfaces. Using a validated sampling and recovery method can mitigate many of these failures. Sampling plans should also take into account the risks of pathogenic viruses where appropriate because bacterial recovery methods will not work.[30] The presence of biofilm-mediated pathogens presents yet another challenge that was assessed as a medium risk failure mode.[13] Interference due to disinfecting solutions use can also be overcome through the use of validated protocols with appropriate robustness.[10]

As noted in Table 2, the use of the other cleaning monitoring methods (ATP bioluminometry and FM) can mitigate the time failure mode and assist in targeting the environmental surfaces that require samples collection for microbial recovery.

Rapid ATP detection

Since use of rapid ATP testing was first suggested, many technical problems have been described with regard to its use within health care settings.[6,31-33] The failure modes identified from our FMEA, which are categorized as medium risk, are noted in Table 2.

The 3 highest RPN and medium risk scores came from unrepresentatively low ATP readings due to a failure of ATP detection.[32] The correlation between high ATP bioluminometry readings and microbes has been established, but there does not appear to be any ATP level (in relative light units) that correlates with nosocomial pathogens at low levels, nor with viruses present on surfaces.[34-37] The lack of correlation with specific pathogens is not recognized as a failure insofar as the setting of an appropriate alert level noting that none of the brands of ATP bioluminometers claim direct correlation with known pathogenic microbes, particularly at low concentrations on surfaces.[8,36,37]

Difficulties interpreting the results of ATP are a medium risk failure mode that underscores the importance of validation and training.[16,31,33]

Mitigations noted against ATP testing are the adjunct use of microbial recovery methods and FM to indicate the frequency of cleaning on relevant environment surfaces, a validated sampling plan, and appropriate levels of training in the use an interpretation of ATP readings.[32]

FM technologies

The data for FM technologies are impressive with excellent correlation with cleaning outcomes.[38] Failure modes noted for were only for unintended or detectable visibility (and preferential removal of FM marker) and for the lack of quantitative information provided through the removal of an FM marker. FM technologies in themselves only measure whether a surface has been attempted to be cleaned, and do not provide an indication of the quantitative hygienic status of a surface. If the FM spot is removed then the data generated are nominal (pass or fail) and are therefore qualitative, although the authors note the highly impressive value of the data and the evidence in favor of this cleaning monitoring method.[4,8,39]

In Table 2, the 2 medium risk failure modes can both be mitigated through the use of alternative cleaning monitoring methods. The visibility of the FM spots is a noted problem, and the additional problem of well-prepared cleaning staff (with their own fluorescent lights to identify the location FM spots) can be mitigated through the sequential or parallel use of rapid ATP detection.[40]

Mitigation

The FMEA assessors considered the failure modes in combination with all 4 cleaning monitoring methods. The failure modes identified for visual inspection were given the classification of high and medium risk, but in each case the use of an alternative rapid method such as ATP bioluminometry could mitigate the risk rating. For microbial recovery, the use of a rapid method of testing, again such as ATP, could work well to mitigate the delay issues, given that correlation has been established with at least high levels of microbial contamination. The classification of medium risk for failure modes of ATP testing can in each case be mitigated through the concurrent use of FM or microbial recovery (during any outbreak of disease). The lack of quantitative information as a failure mode for FM can be mitigated easily through adjunct use of ATP.

Figure 1 outlines a new theoretical approach that attempts to overcome the identified failure modes of the cleaning monitoring methods by using the strengths of 1 method to cover the weaknesses of another method. Figure 1 demonstrates the potential synergy provided by using the cleaning monitoring methods concurrently and in sequence, rather than in isolation or in a parallel trial. When considered together as a bundle of solutions for cleaning monitoring, the 4 methods work best in combination, with

Table 2
Fifteen failure modes ultimately found to be associated with the highest risk priority numbers (RPN) and associated failure risk

Cleaning Monitoring method	Failure mode	Frequency*	Severity*	Detectability*	RPN†	Risk classification‡	Mitigation suggestions
Visual Inspection	Surface looks clean but is contaminated with pathogenic microbes[11]	3	3	3	**27**	High	Testing of surfaces with a quantitative method for determining surface hygiene may indicate contamination (eg, ATP testing)
	Surface looks clean but is contaminated with non-visible soils[6]	3	2	3	**18**	High	Testing of surfaces with a quantitative method for determining surface hygiene may indicate contamination (eg, ATP testing)
	Surface looks clean but has not been subject to routine cleaning processes[12]	2	2	3	**12**	Medium	Use of an FM technology will indicate surface cleaning frequency rate. ATP testing alone will not be sufficient to indicate cleaning frequency
Microbial recovery	Result from sampling is delayed by 48 h or more[27]	3	3	2	**18**	High	Testing of surfaces with a rapid method (eg, ATP testing) can rapidly inform the sampling processes by indicating any loci of contamination
	Pathogenic bacteria are not detected on swab but are present on high-touch obejects[10]	2	2	3	**12**	Medium	Validation of sampling methods with examination of different methods results can improve microbial recovery
	Viruses (pathogenic) are not detected by sampling methods[30]	2	3	2	**12**	Medium	Selection of a virus-sensitive sampling and recovery method is required where cross-infection with viruses is a known risk factor
	Pathogenic bacteria are not detected due to poor environmental sampling plan or methodology[11]	2	2	2	**8**	Medium	Validation of sampling methods with examination of the effect of different methods can improve microbial recovery. Adjunct use of rapid ATP detection can assist in identification of possibly contaminated environmental locations
	Pathogenic bacteria are present in biofilms that are not recovered in normal sampling[13]	2	2	2	**8**	Medium	Swabbing techniques should utilize different approaches to dislodge resident organisms. Subsequent recovery techniques may be required for cultures to adjust for slower recovery dynamics
ATP bioluminometry	Failure to detect the ATP present on contaminated surfaces (possible lysis failure by ATP consumables-reagent)[32]	2	2	3	**12**	Medium	Microbial recovery can be used for confirmatory quality control where FM indicates inadequate cleaning but low ATP readings do not reflect inadequate cleaning. A validated sampling plan will ensure testing is representative
	ATP swab fails to pick up sufficient bacteria to indicate surface contamination (falsely low ATP reading)[32]	2	2	3	**12**	Medium	Microbial recovery can be used for confirmatory quality control where FM indicates inadequate cleaning but ATP readings are in the low range and biofilm is suspected
	Failure to detect viruses due to absence of ATP[32]	3	2	2	**12**	Medium	Use of an FM technology is low cost and will indicate surface cleaning frequency in addition to use of rapid ATP detection. A suitable microbial recovery method for virus detection could also be used
	Similar before-and-after ATP readings that do not indicate that cleaning has or has not occured[31]	2	2	2	**8**	Medium	Rapid ATP detection requires a carefully considered sampling plan to ensure that multiple readings are taken for proper interpretation of the results. Adjunct use of FM will indicate if cleaning has occurred
	Incorrect interpretation of the ATP readings from the hospital surfaces[33]	2	2	2	**8**	Medium	ATP readings are subject to both variability and sampling error. Careful validation of the sampling plan and use of ATP will reduce both types of error. Adjunct use of FM will indicate cleaning. Training in reading ATP results is essential

FM technologies	FM spots are visible to cleaning staff and are preferentially removed[40]	2	3	2	**12**	Medium	Testing of surfaces prior to use of FM will indicate surface suitability for use of FM
	FM assessment does not indicate a quantitative level of cleanliness has been achieved by the cleaning process, even when the cleaning process is conducted as intended[8]	3	2	2	**12**	Medium	Validation of the cleaning processes should be conducted to ascertain the quantitative contribution of the cleaning method to the primary purpose of soil removal as a primary aim. ATP measurement will provide an adjunct measurement of surface cleanliness that is semiquantitative

FM, fluorescent marker.
*1 = low, 2 = medium, and 3 = high.
†RPN = Frequency × severity × detectability.
‡High = RPN 15, medium = RPN > 8 < 15, and low = RPN < 7.

system redundancy built in to the process of monitoring through carefully considered and adjunct use of each of the available methods.

The costs attached to cleaning monitoring method are a substantial concern, but these do vary by geographic region, commercial availability, and local practices. The individual cost components on any 1 cleaning monitoring method are outside of the range of this study and will require further investigation and quantitation. The opportunity for cost-efficiency in the mixed monitoring approach outlined in Figure 1 should be further investigated to find the balance between cost and efficiency in the supervision of cleaning services.

In an integrated study using the 4 monitoring systems in parallel, costs could be managed in a very different manner to suit the location of the monitoring and the specific infection control circumstance.

DISCUSSION

The worst of all failure modes observed from the 4 cleaning monitoring methods was for visual inspection.[6] The use of microbial recovery through environmental sampling has a major failure mode due to the delay in receiving results.[10] Clearly FM is a simple tool that provides excellent quality data on the frequency of noncleaning; that is, the qualitative question of how often the cleaning is not conducted as intended.[38] There is also a need for a monitoring process that has the role of quantitation of cleaning performance in terms of surface cleanliness, and rapid ATP testing could be developed to fill this void, subject to interpretation and validation issues.[8,31-36]

Not 1 of the current cleaning monitoring methods could overcome all of the potential failure modes that were identified for that monitoring method when considered in isolation.

When the cleaning monitoring was considered in a single flowchart (Fig 1), it became clear that use of the monitoring methods in combination offered the opportunity to mitigate the effect of failure modes through the synergistic value of the cleaning monitoring methods were used both in parallel and in sequence. The strength of visual inspection was maximized, whereas the high-risk failure modes are mitigated through use of both FM and ATP bioluminometry. A highly targeted (adjunct) use of microbial recovery, particularly during an outbreak, can be used to provide quantitative information against any specific pathogens of concern where timely feedback is less of a concern.[27,28,37]

When using both FM and ATP testing for cleaning monitoring, health care providers must set a suitable acceptable level of pass or fail using the data generated.[8] For example, FM will indicate the frequency of cleaning for surfaces and infection control professionals (ICPs) should consider the acceptable level of success before any intervention on the quality of the cleaning service provided.[4] Similarly for rapid ATP, the appropriate level of pass or fail for the brand of ATP device should be carefully considered before implementation of use of rapid ATP detection, so that intervention on the quality of cleaning service provided is appropriate.[36]

The issue of cost of monitoring is the subject of ongoing studies, and costs will vary by location and commercial issues. The outline considered in Figure 1 demonstrates that by using an integrated approach, costs of monitoring can be managed to achieve the lowest potential cost to yield the greatest likelihood of successful cleaning performance.

This work is intended as an exemplar on the use of FMEA, and is by no means fully comprehensive. The failure modes identified by our FMEA assessors are subjective and may not fully reflect all of the potential failures in cleaning monitoring methods. However,

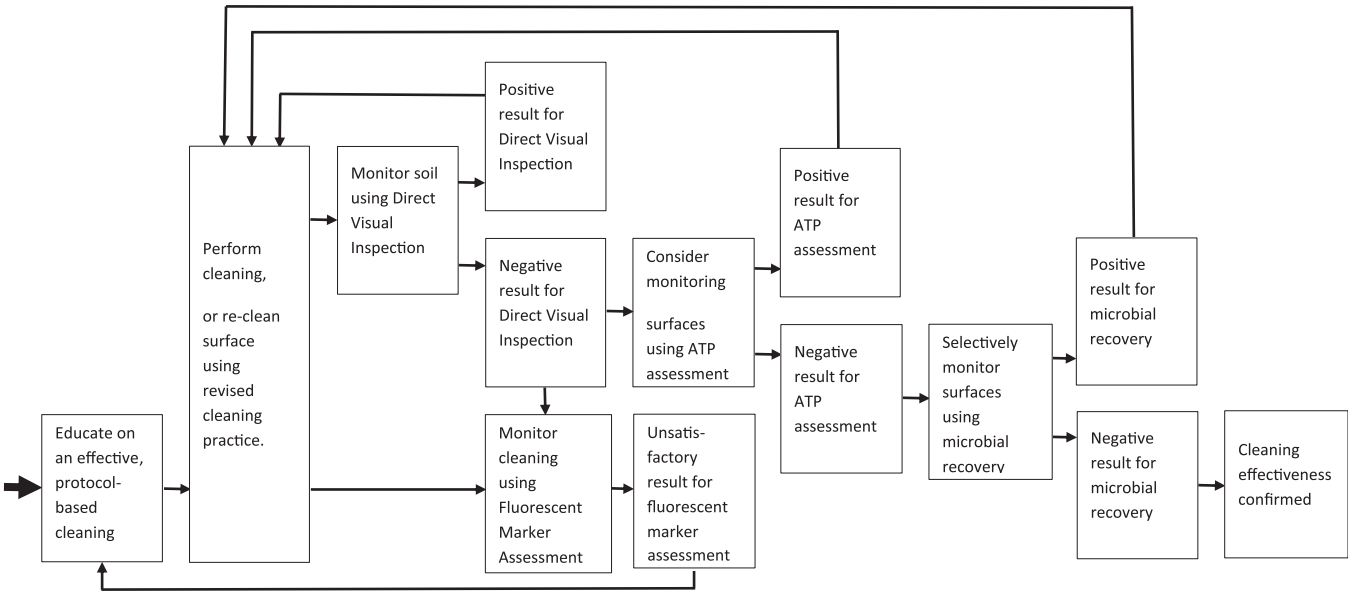

Fig 1. Model for cleaning monitoring.

the work in reconstructing the approach of cleaning monitoring using all 4 available methods does offer ICPs a new approach to drive quality improvements in environmental cleaning processes through more reliable data collection in ongoing studies. Further studies are underway to investigate and confirm the findings of this FMEA-based risk assessment study.

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Chapter 8

The perennial problem of variability in adenosine triphosphate (ATP) tests for hygiene monitoring within healthcare settings

ORIGINAL ARTICLE

The Perennial Problem of Variability In Adenosine Triphosphate (ATP) Tests for Hygiene Monitoring Within Healthcare Settings

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OBJECTIVE. To investigate the reliability of commercial ATP bioluminometers and to document precision and variability measurements using known and quantitated standard materials.

METHODS. Four commercially branded ATP bioluminometers and their consumables were subjected to a series of controlled studies with quantitated materials in multiple repetitions of dilution series. The individual dilutions were applied directly to ATP swabs. To assess precision and reproducibility, each dilution step was tested in triplicate or quadruplicate and the RLU reading from each test point was recorded. Results across the multiple dilution series were normalized using the coefficient of variation.

RESULTS. The results for pure ATP and bacterial ATP from suspensions of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* are presented graphically. The data indicate that precision and reproducibility are poor across all brands tested. Standard deviation was as high as 50% of the mean for all brands, and in the field users are not provided any indication of this level of imprecision.

CONCLUSIONS. The variability of commercial ATP bioluminometers and their consumables is unacceptably high with the current technical configuration. The advantage of speed of response is undermined by instrument imprecision expressed in the numerical scale of relative light units (RLU).

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The original suggestion to use rapid adenosine triphosphate (ATP) bioluminometers to monitor surface hygiene has yet to see practical fulfilment among healthcare infection control and prevention practitioners.[1] Rapid ATP bioluminometry has been shown to reliably distinguish between 10-fold serial dilutions of various common bacterial species.[2,3] ATP devices also produce a nearly instantaneous measurement of surface cleanliness, which has been suggested to provide useful feedback for quality improvement of cleaning services.[4]

Currently, authoritative sources have declined to endorse ATP testing on a stand-alone basis for monitoring surface hygiene in healthcare settings.[5–7] While the detection of ATP on different surfaces has been validated with multidrug-resistant organisms (MDROs) where a large amount of contamination is present, little correlation has been made using ATP measurements when small amounts of infectious contaminants are present.[8,9]

Variability has been identified as a concern in the use of handheld ATP bioluminometers,[10,11] and 3 particular aspects are troublesome. First, the ATP reading scale known as relative light units (RLUs) lacks quantitation against a known standard and therefore cannot be calibrated, coincidentally diminishing interbrand comparability.[12]

Second, as we have shown previously, this high variability leads to poor precision (repeatability) at a magnitude that prevents ATP devices from adequately distinguishing between similar ATP concentrations or similar levels of contamination.[12] In this earlier study, the presence of raw data 'outlier' readings was unpredictable and nominally undetectable to those using an ATP device, and these outliers can occur both above and below the interquartile range.[12] This variability can cause unexpected and unusual results, such as ATP readings that are higher following cleaning than prior to cleaning.[13]

Third, the impact of this variability is greatest at the measurement level that defines the boundary between acceptable and unacceptable cleanliness. This criterion has been suggested through various studies at the lower end of the RLU reading range, between 500 RLU and 100 RLU, and is dependent upon the brand of ATP bioluminometer.[14,15] Attempts to correlate proposed cleaning standards with benchmarks expressed in RLUs, at the low RLU levels required for hospital hygiene, have been manifestly unsuccessful.[16–19] Even the applications of a method utilizing a receiver operating curve have not resulted in a widely accepted definitional boundary for an ATP-based cleaning benchmark.[15,20]

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Uncontrolled variability leading to poor precision is a major problem for statistical methods comparing differing treatment or control groups.[21] The risk of type I and type II errors in environmental monitoring is increased if the devices are subject to substantial variability.[22] These type I and II errors using handheld ATP bioluminometers can present as false-positive or false-negative results, generating additional costs either way. Variability in results from ATP bioluminometers is the most recurrent technical concern regarding their use in cleaning monitoring studies using quasi-experimental frameworks.

Another particular concern with portable ATP devices is the risk of random error, which is undetectable in single monitoring samples.[23] Most studies have been focused on the risks of systemic error through reading inaccuracy over a wide range of dilutions; however, the magnitude of random error and accompanying dispersion of the data set (ie, RLU readings) has confused many studies.[2,3] The issues of systematic error with ATP devices are not considered in this paper.

In this study, a continuation of our work, 4 commercial brands of rapid ATP bioluminometers were tested to validate their performance in a series of controlled experiments. The aim of this study was to assess the precision of and reproducibility of results from the ATP devices to qualify their role in cleanliness monitoring. The critical aspects of this study were the evaluation of the precision of the ATP measurements as expressed in RLUs and the assessment of the magnitude of random error using both pure-source ATP and bacterial ATP.

This paper outlines our findings regarding variability for all of the ATP devices we tested under controlled conditions.

METHODS

The 4 commercial brands of ATP bioluminometers and their consumables were considered in this work: 3 M (Cleantrace), Kikkoman (Lucipac-pen), Hygiena (Ultrasnap), and Charm (Pocket Swab Plus). This series of experiments investigated the response of each brand of ATP device across dilution series to known ATP quantities and to various bacteria in suspension cultures through direct application to swabs.[12] This method of application removed swab technique as a variable, thus exposing more clearly the underlying variability of the ATP devices. The use of multiple repetitions of dilution series established a practical dynamic range of concentrations for each of the ATP bioluminometers, both for a known source of ATP and for the ATP of bacterial species included in the studies. Each ATP device and its consumables were used in accordance with the respective manufacturer's instructions, including refrigerated storage, use at room temperature, and consumables used prior to the expiration date.

Testing Using Quantitated ATP

Our study used a known source of ATP (Sigma Aldrich, Castle Hill, Australia) to assess ATP device response; while the quantitative aspects of the concentration of the ATP were confirmed using high-performance liquid chromatography (HPLC; Shimadzu, Japan) in a validated method. The use of a fully validated, laboratory HPLC allowed a defined quantity of ATP to be used on each sample, thus anchoring the experimental results and qualifying the confusing RLU scaling to minimize the risks of random and/or systematic errors in measurement. The method of application used 10 µL of each concentration of ATP solution applied directly to each swab using a calibrated pipette.[12]

At each dilution point, replicated testing with a minimum of 3 swabs was used for each ATP bioluminometer, and wherever possible, test points were sampled in quadruplicate. For each dilution series, the experiment was repeated at least twice, using multiple batches of consumables from each of the ATP device suppliers.

The ATP solutions were tested in both a 10-fold dilution series and in a number of 20% dilution series at various concentrations of ATP. In total, >840 swabs were used to sample >200 concentrations. The Charm device and consumables were obtained toward the end of the study, so the number of sample concentrations and total number of swabs used to test that brand were smaller than for the other three brands.

Microbiological Testing

The experiments using bacterial-suspension cultures were each repeated on multiple occasions. At each concentration in the dilution series, testing was conducted in quadruplicate, and medians have been used to present typical responses.

The bacteria used in the experiments were *Staphylococcus epidermidis* (ATCC 12228) and *Pseudomonas aeruginosa* (ATCC 15692). All were tested as suspension cultures. While the strains tested are not regarded as intrinsically invasive, both *S. epidermidis* and *P. aeruginosa* have been associated with implanted device failure (*S. epidermidis*), multi-drug-resistant organisms (*P. aeruginosa*), and healthcare-associated infections.[24,25]

Staphylococcus epidermidis and *P. aeruginosa* were enumerated by dilution series onto nutrient agar–spread plates (Oxoid, Thermo Scientific, Waltham, MA) incubated at 37°C for 48 hours. The *P. aeruginosa* plates were read at both 24 hours and 48 hours. The quantitative enumeration of the suspension cultures allowed for quantitation of the results and also minimized the risks of systematic error from RLU measurements.

Phosphate-buffered saline solution (PBS; Univar, Sydney, Australia) was used for the dilution series at 10 mMol/L and buffered to pH 7.0 The solution was freshly made and sterilized with each experimental round. During an early experimental round, dilutions prepared with tryptone soy broth (TSB) were found to interfere with one of the ATP bioluminometers (Kikkoman), and the use of TSB was discontinued thereafter. This TSB interference observation was

confirmed subsequently with separate laboratory operators in an independent test run.

Tests were conducted in 10-fold dilution series and a number of 20% dilution series at various concentrations of suspension culture. All test concentrations were tested in quadruplicate; however, in some instances samples were measured in triplicate. Testing against bacterial cultures was conducted by direct application in the same way ATP was tested, with 10 μL applied per swab. In this study, we used >1200 ATP swabs and ~300 individual concentrations of bacterial suspensions.

Statistical Approach

Because the ATP bioluminometer readings in RLU were not comparable between brands, absolute measures of random error, such as standard deviation, were not directly comparable between brands. The standard deviation increased with increases in the underlying value of tested dilutions, so another statistical measure was required.[26] To assess the relative size of the random error, measurements were normalized using the coefficient of variation (CV), which is the ratio of standard deviation to the mean.[27] This method is particularly helpful with non-symmetrical data.[28]

This normalized measurement allowed the comparison of instrument precision among brands at varying concentrations. A CV of 0.4 implies the average distance between observations at a single concentration for that ATP device; the mean of those observations is 40% (0.4) of that mean. Alternatively, if the mean was equal to the absolute truth of the measurement for that ATP device, the expected error (or precision in this case) would be ~0.4 times the mean, but according to Reed et al,[27] a 1 in 5 chance remains that the reading will be incorrect (imprecise) by a factor of 2 or more.[27] The higher the CV, the lower the precision of the equipment.

The replicated data from the experiments were used to obtain the individual CV for each set of readings for each device at each concentration point, which captured the precision in a highly variable data set with these rapid ATP bioluminometers.[10,29] The use of CV as statistical approach can also capture both the random error and some of the systematic errors, which directly affects the precision of ATP measurements.

The distribution of CVs is summarized in Figures 1–3 using boxplots. In the plots, the central line in each box represents the median value, with a box drawn around the second and third interquartile ranges. The whiskers (the lines extending both below and above the box) represent the tenth and ninetieth percentiles of the range; the dots above and below represent values that are outside the expected spread of results (ie, outliers).

The regularity of outliers both above and below the interquartile ranges of samples was noted as a problem early in these studies. Arithmetic median was used rather than averages to remove the distortion caused by outliers in raw data sets. When considering dynamic range, however, this sampling approach is not always available for use in field-based measurements. The CV method was preferred to signal-to-noise ratio ($\bar{x}/\sigma$) due to the large volume of random error.

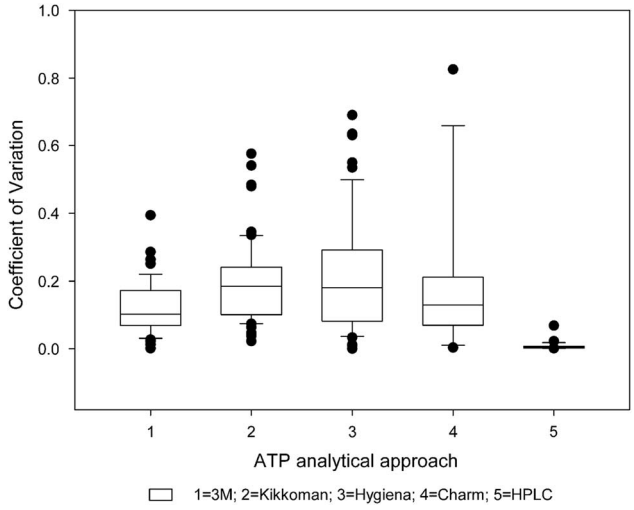


FIGURE 1. Coefficient of variation for 4 ATP bioluminometers using ATP testing.

All calculations were conducted and graphs were produced in Microsoft Excel 2010 (Microsoft, Seattle, WA), and where required, these calculations were confirmed using Sigma Plot (Systat, San Jose, CA) or SPSS (IBM, NY).

RESULTS

Variability measures for all 4 bioluminometers were standardized using the coefficient of variance (CV). The 3 testing platforms for the ATP bioluminometers and their consumables first quantitated pure-source ATP (Figure 1) then the ATP of the 2 bacterial species *S. epidermidis* (Figure 2) and *P. aeruginosa* (Figure 3). The variability expressed as CV was present across the full dynamic range for all ATP bioluminometer brands tested and was not concentration dependent.

Figure 1 indicates the CV present in all 4 ATP bioluminometers and displays the contrast of very low CV calculated for the HPLC. HPLC measurements, taken in triplicate, demonstrated a correlation of $r = .998$, indicating the high precision and low variance expected of a fully calibrated precision laboratory instrument. When compared with the HPLC values, all 4 of the ATP devices indicated higher levels of variance, and 2 of the devices (Kikkoman and Hygiena) produced CV > 0.4. In a prior study, also using known-source and quantitated ATP, the 3M device also produced CV = 0.4.[10]

Figure 2 shows the CV for all 4 ATP bioluminometers when measured against quantitated dilution series using the Gram-positive bacterium *S. epidermidis*. Figure 2 indicates that all 4 ATP bioluminometers scored a CV > 0.4 and 2 of the devices (Kikkoman and Charm) showed CV > 1.0 ($\sigma > \bar{x}$).

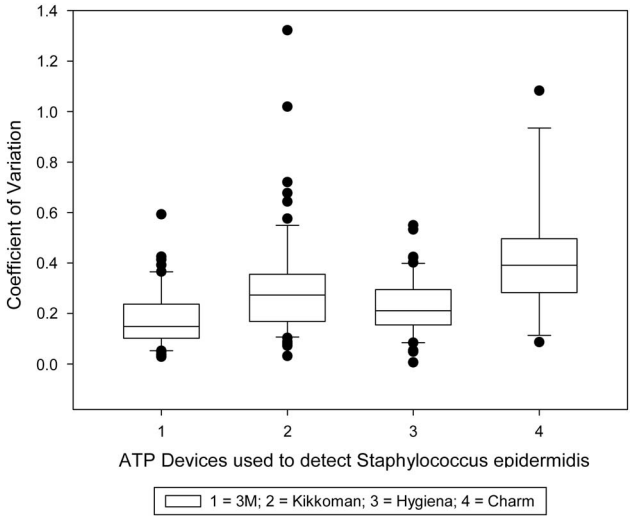


FIGURE 2. Coefficient of variation for 4 ATP bioluminometers using *Staphylococcus epidermidis.*

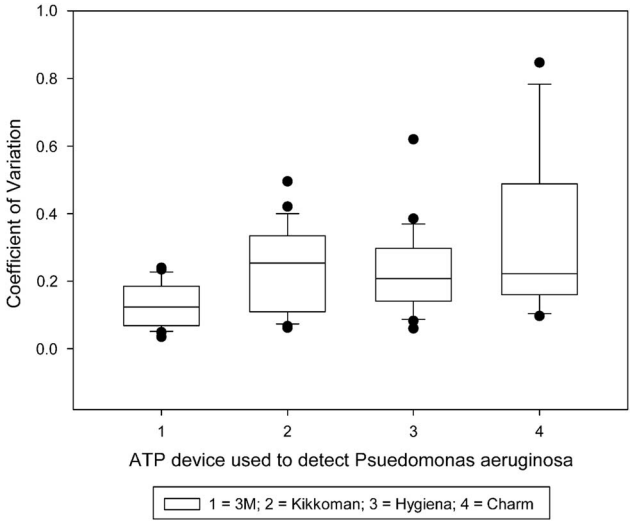


FIGURE 3. Coefficient of variation for 4 ATP bioluminometers using *Pseudomonas aeruginosa.*

Figure 3 indicates the CV for all 4 ATP bioluminometers when measured against quantitated dilution series' using the Gram-negative bacterium *P. aeruginosa*. Figure 3 clearly indicates the same pattern of CV shown in Figures 1 and 2.

The experimental series used >500 sample concentrations and >2,000 swabs with robustly defensible and repeated outcomes.

Figure 4 shows the overall spread for the 4 devices with the range (high–low) and median indicated for each ATP bioluminometer brand and each material tested. The median readings of all 4 ATP devices against the 3 validated soiling materials (pure-source ATP and 2 bacteria) yielded CVs clustered between 0.0995 and 0.39. As shown in Figure 4, all 4 brands produced at least 1 high-range value (CV > 0.5), which undermines confidence in instrument precision for field use, particularly where surface sampling uses only a single swab.

DISCUSSION

The use of rapid ATP testing in the monitoring of environmental cleanliness in healthcare settings has the advantages of quick response, semi-quantitative evaluation, ease of use, and immediate feedback regarding cleaning performance outcomes for surface cleaniness.[4,6] Rapid ATP monitoring also has identified flaws that require careful consideration when designing a sampling regimen.[9,11,29]

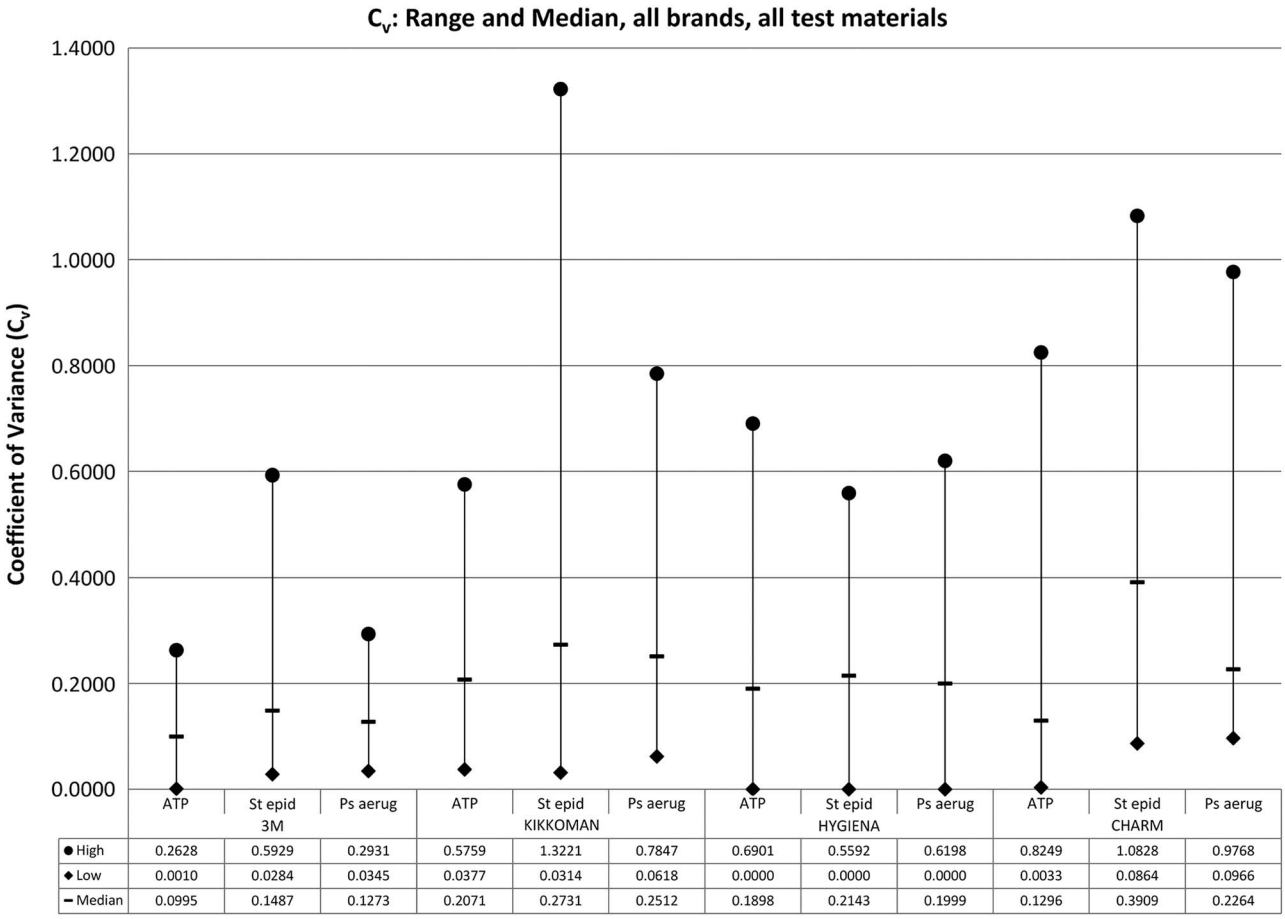
A high level of variability using rapid ATP bioluminometers was observed across all of the brands tested with CV results; similar results were acquired between brands for both quantitated ATP and bacterial suspension cultures. The lack of precision and lack of reproducibility when using commercial rapid ATP bioluminometers undermines both the validity of the results and the confidence with which the devices can be used, particularly in busy healthcare environments with cost constraints on sampling. Furthermore, variability in RLU readings increases the likelihood of both false-positive and false-negative outcomes from studies in which metering variation is not considered prior to interpretation of results.

In healthcare, complexity in the management of cleaning is compounded by the irregular distribution of microorganisms despite recognition of some location patterns.[30,31] The presence of dry-surface bacterial biofilms containing multidrug-resistant organisms adds another level of uncertainty in the monitoring of cleanliness.[32] The accurate measurement of cleanliness failure is essential for infection prevention, and if measurement is accurate and precise, the sampling method can be minimized as a confounder in underestimating the presence of pathogenic contaminants.[33]

In cleaning monitoring, the use of an environmental sampling method can lead to both type I and type II statistical errors. The dangers of a false-positive RLU reading (type I error) may be the cost of additional cleaning. This cost could be significant if the cleaning benchmark is set unreasonably low or if the ATP bioluminometer overstates the level of contamination due to random error. Importantly, false-negative test results with inaccurately low RLU readings (type II error) compound the potential infection risks from insufficiently cleaned surfaces.

Quality assurance is compromised when a device such as an ATP bioluminometer lacks the precision required to reliably articulate cleanliness as intended by its use. Reliability in the precision of RLU readings is particularly important at the low end of the dynamic range where the correlation between ATP detection and the presence or absence of pathogenic microbes is most difficult to determine.[8]

One way to reduce variability is to address the RLU scaling, which is highly relative and is different for each brand of ATP device.[12] An interim way to manage the impact of variability



	ATP	St epid 3M	Ps aerug	ATP	St epid KIKKOMAN	Ps aerug	ATP	St epid HYGIENA	Ps aerug	ATP	St epid CHARM	Ps aerug
● High	0.2628	0.5929	0.2931	0.5759	1.3221	0.7847	0.6901	0.5592	0.6198	0.8249	1.0828	0.9768
◆ Low	0.0010	0.0284	0.0345	0.0377	0.0314	0.0618	0.0000	0.0000	0.0000	0.0033	0.0864	0.0966
– Median	0.0995	0.1487	0.1273	0.2071	0.2731	0.2512	0.1898	0.2143	0.1999	0.1296	0.3909	0.2264

FIGURE 4. Coefficient of variation: range and median for all brands tested and all test materials.

with ATP devices is to manage the sampling plan through the sequential and parallel use of different methods of cleaning monitoring.[34]

The results presented here were obtained with the advantages of controlled conditions, multiple repetitions, large numbers of swabs used in a wide array of concentrations across the entire reading range of the devices. Unfortunately, this strictly controlled approach probably underestimates the level of variation that occurs in uncontrolled field-based studies; for example, swab technique adds additional variability. A shortfall of this study was that just 1 ATP device per brand was used in the experiments. Purchased consumables (albeit used according to manufacturer recommendations) lack consistency that may not be reflected in manufacturer-supported studies with fresh consumables and strict batch controls. However, this work does represent the normal experience of field use, suggesting that all brands of ATP devices exhibit similar variability and lack of precision.

Furthermore, in this study, more repetitions may have decreased the range of CV results, thus bringing the mean and median into closer alignment. However, the range data (Figure 4) would be unaffected.

Rapid ATP measurement using handheld devices is quick and relatively inexpensive, but caution is required in the interpretation of results. As ATP devices are currently configured, the inherent variability and lack of precision in the results obtained may lead to erroneous conclusions; thus, data collected using these devices and the conclusions stemming from them warrant careful consideration.

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Chapter 9

A pilot study into locating the bad bugs in a busy intensive care unit

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Major article

A pilot study into locating the bad bugs in a busy intensive care unit

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Key Words:
MDRO locations
ATP correlation
Dry surface biofilm
Intensive care unit
Staff work area
High-touch objects

Background: The persistence of multidrug-resistant organisms (MDROs) within an intensive care unit (ICU) possibly contained within dry surface biofilms, remains a perplexing confounder and is a threat to patient safety. Identification of residential locations of MDRO within the ICU is an intervention for which new scientific approaches may assist in finding potential MDRO reservoirs.

Method: This study investigated a new approach to sampling using a more aggressive environmental swabbing technique of high-touch objects (HTOs) and surfaces, aided by 2 commercially available adenosine triphosphate (ATP) bioluminometers.

Results: A total of 13 individual MDRO locations identified in this pilot study. The use of ATP bioluminometers was significantly associated with the identification of 12 of the 13 individual MDRO locations. The MDRO recovery and readings from the 2 ATP bioluminometers were not significantly correlated with distinct cutoffs for each ATP device, and there was no correlation between the 2 ATP devices.

Conclusion: The specific MDRO locations were not limited to the immediate patient surroundings or to any specific HTO or type of surface. The use of ATP testing helped rapidly identify the soiled locations for MDRO sampling. The greatest density of positive MDRO locations was around and within the clinical staff work station.

Pathogenic microorganisms are a persistent risk to patient safety in modern health care settings, particularly within active intensive care units (ICUs).[1] The presence of multidrug-resistant organisms (MDROs) embedded within dry surface biofilms adds further complexity to the cleaning process.[2] A validated scientific approach to evidence-based cleaning requires a multidisciplinary understanding of all intersecting parts of the hygiene puzzle.[3]

The location of MDROs within an ICU may be subject to patterns of localization and dispersion.[4] There is an expected "hot zone" of bacterial surface contamination normally focused around the bed of an infected patient.[5,6] The transfer of these MDROs away from the patient area and throughout the ICU is thought to occur largely via unwashed hands and gloved hands.[7,8] High-touch objects (HTOs) are located throughout clinical areas and can provide a likely transit point for microbes owing to the frequency of hand touches.[9]

A lack of cleaning in the clinical workspace, which is distant from the direct patient surroundings, may allow biofilm development without mitigation.[10] This opens the potential for MDRO to move seamlessly between residential biofilm locations either close to or away from the patient, and then back and forth via hands onto surfaces and objects that are frequently touched within the ICU.[11] The identification of residential biofilm locations thus becomes a critical requirement in the validation of any cleaning process within health care settings and particularly in ICUs.[12]

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The use of adenosine triphosphate (ATP) for monitoring of cleaning has been shown to be superior to visual inspection of cleaning performance.[13] Various studies have indicated that ATP devices can detect and reliably indicate high readings in relative light units (RLU) when high numbers of bacteria or ATP sources are present.[14-16] Recent studies have further indicated that despite the lack of species identification, commercial ATP devices may aid the detection of MDRO species on surfaces.[17] The use of commercial ATP devices in ICU settings has been shown to add limited improvement to cleaning validation within ICUs.[18,19]

The poor level of precision with the current generation of ATP meters requires careful consideration in the methodology of any sampling plan.[20] Field trials using different brands have not shown any definitive correlation either with cleaning or between brands.[21-23] The use of overlapping monitoring approaches has been suggested as a method to overcome individual weaknesses in monitoring systems.[24]

In the present study, conducted within an active ICU, a series of environmental cleanliness assessments were conducted to identify locations of MDROs. Microbial sampling was also conducted to confirm the presence and location of MDROs and likely hotspots for the presence of dry surface biofilms. Two commercially available ATP devices were evaluated to determine whether they could be reliably used to indicate soiled versus relatively clean surfaces as a rapid indicator for prospective sampling locations.

METHODS

In this pilot study, 3 separate sampling runs were conducted. All sampling sites were identified as HTOs that have been previously reported in the literature.[2,9,10,25] The first sampling run was performed by a single operator only obtaining microbial swabs using the method described below. On the second and third sampling runs, 2 operators sampled various HTO locations, performing microbiology and ATP sampling. The full set of sample locations is listed in Table 1.

Environmental swabs were taken using a sterile gauze cloth soaked in a 0.9% sodium chloride solution, swabbed onto the surface using sterile tweezers, and then immediately immersed in sterile tryptic soy broth (TSB).[26] The gauze swabs were then incubated at 37°C for 24 hours and tested for the presence of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and multidrug-resistant gram-negative bacteria by spread plating onto methicillin-resistant *S aureus* and vancomycin-resistant enterococci chromogenic media (bioMérieux, Marcy-l'Étoile, France), extended-spectrum beta-lactamase chromogenic media, and horse blood agar (Edwards, Sydney, Australia). Potential MDRO growth was identified and tested for antibiotic susceptibility via matrix assisted laser desorption ionization time-of-flight (VITEK automated microbial identification system; bioMérieux).

The 2 commercially available ATP bioluminometers and their consumables selected for the study were the Kikkoman ATP device with Lucipak-Pen swabs (Foodcare Systems, Leeton, Australia), and the Hygiena ATP device with Ultrasnap swabs (Key Diagnostics, Lane Cove, Australia). All consumables were stored in accordance with the manufacturers' instructions. ATP swabs were taken at each identified location first with the Hygiena device and then with the Kikkoman device. The final swab taken at each location was for the microbial recovery of MDROs as described above.

The first sampling run used only environmental swabs on likely HTOs. Sampling sites for the second and third runs were selected after the first run. The second run used both nonpaired and a paired combination of the 2 ATP devices with microbial sampling. The selection process for the second sampling run was neither well matched nor randomized. The third run used a matched-paired approach with both ATP devices and microbial sampling at each sample location.

Statistical analyses were performed using both Excel 8.0 (Microsoft, Redmond, WA) and Sigma Plot 12.0 (Systat, San Jose, CA). Tables and Figures were drawn using Microsoft Excel 8.0. The χ^2 calculations were manually rechecked and corrected using the Yates correction (for low sample numbers). The Yates correction used 1 degree of freedom (df) in the calculation $df = (r-1)(c-1)$, where r represents rows and c represents columns.[27] Fisher's exact test was calculated using Sigma Plot.

RESULTS

A total of 62 environmental swabs were obtained from the 3 sampling runs (23 from the first run, 16 from the second run, and 23 from the third run). The first sampling run collected microbial swabs from 23 separate HTO locations. The second sampling run examined 29 separate HTO locations, using a combination of 16 environmental swabs and 43 ATP swabs (28 Hygiena and 15 Kikkoman). During the second sampling run, only 13 of the 29 locations had the environmental swabs matched with the use of both brands of ATP swabs. The third sampling run used a fully matched approach, in which all 23 sites examined were subject to 23 environmental swabs and 23 of each of the branded ATP swabs (46 swabs). Table 1 lists the sampling locations and methods.

The first sampling run collected 23 microbiology swabs using a single operator, without ATP testing. From this first sampling run, a single MDRO-positive sample was confirmed from a patient folder within the immediate patient zone (MDRO recovery rate of 4.3%; $n = 23$). The time interval from sampling to MDRO confirmation was longer than 72 hours.

The second sampling run introduced the use of ATP testing. The results of ATP tests are expressed in RLU. The nearly immediate RLU readings were accepted to indicate the general level of soiling in each sample location.[14-16] Many of the sampling locations with relatively low RLU were not sampled for either MDRO or for the 2 branded ATP tests. This sampling approach limited the power of findings from this pilot study and introduced the potential for sampling error, particularly with insufficient replicates from each sample location.[28] The rise in ATP levels in this second sampling run, as sampling locations moved from the immediate patient zone toward the clinical workstation, is illustrated in Fig 1.

This second sampling run examined a total of 29 individual HTO locations. Environmental swabs were taken on all surfaces with relatively higher RLU readings. Sixteen locations were swabbed using environmental swabs; 3 of the 16 swabs were taken using only a single branded ATP reading (all Hygiena). All of these 13 locations were examined with environmental swabs and using a paired approach using both brands of ATP tests. From the 13 swabs with matching pairs, 5 of the environmental samples were later confirmed (>72 hours) as positive for an MDRO. From this second sampling run, only the 13 matched pairs were included in the statistical analysis.

The third sampling run sampled 23 locations focused around the clinical workstation, with all samples paired (both ATP devices and microbial swabs). Seven of the 23 locations were confirmed as positive for a known MDRO. Several of the MDRO-positive items were later removed for further examination for microscopic investigation of dry surface biofilms.

The HTO sample locations that were positively identified with MDROs during the 3 sampling runs are listed in Table 2. Terminal cleaning was performed in the patient surroundings after evacuation of any patient from the individual bed bay during each of the 3 sampling runs.

The combination of the paired testing in sampling runs 2 and 3, where the data were matched with both environmental microbial

Table 1
Sampling locations and methods used for each sampling run

HTO	First sampling run	Second sampling run	Third sampling run
Signage on patient chart table	Micro only		
Pathology folder on patient chart table	Micro only		
Cord holding MSDS in bedpan room	Micro only		
Isolation room (MRSA patient), supply table, top		ATP (Hyg and KK), micro	
Isolation room (MRSA patient), chair, top		ATP (Hyg)	
Isolation room (MRSA patient), chair, seat		ATP (KK)	
Isolation room (MRSA patient), bed railing		ATP (Hyg and KK), micro	
Isolation room (MRSA patient), IV pole		ATP (Hyg)	
Isolation room (MRSA patient), IV device top		ATP (Hyg), micro	
Isolation room (MRSA patient), IV pump face		ATP (Hyg), micro	
Bedside monitor		ATP (Hyg)	
Patient chart table top		ATP (Hyg)	
Patient chart table, bulldog clip		ATP (Hyg and KK), micro	
Patient supply table, top		ATP (Hyg and KK), micro	
IV pole		ATP (Hyg and KK), micro	
Bedside blood pump, face of device		ATP (Hyg and KK)	
Bedside oxygen pump, face of device		ATP (Hyg)	
Patient bathroom, soap dispenser button		ATP (Hyg)	
Patient bathroom, door handle		ATP (Hyg)	
Bedpan room, door handle		ATP (Hyg)	
Bedpan room, bedpan washer handle		ATP (Hyg), micro	
Bedpan room, boxes or crates (2 items)		ATP (Hyg and KK), micro	
Bedpan room, inside storage box		ATP (Hyg)	
Clinical work station, arterial blood gas machine		ATP (Hyg and KK), micro	
Clinical work station, telephone		ATP (Hyg and KK), micro	
Clinical work station, NUM clipboard		ATP (Hyg and KK), micro	
Clinical work station, storage room door handle		ATP (Hyg)	
Clinical work station, crash cart handle		ATP (Hyg)	
Clinical work station, keyboard and mouse		ATP (Hyg and KK), micro	6 samples: ATP (Hyg and KK), micro
Clinical work station, crash cart clipboard		ATP (Hyg and KK), micro	11 samples: ATP (Hyg and KK), micro
Clinical work station, chair		ATP (Hyg and KK), micro	6 samples: ATP (Hyg and KK), micro

Hyg, Hygiena; *KK*, Kikkoman; *micro*, microbial sampling; *NUM*, nurse unit manager.

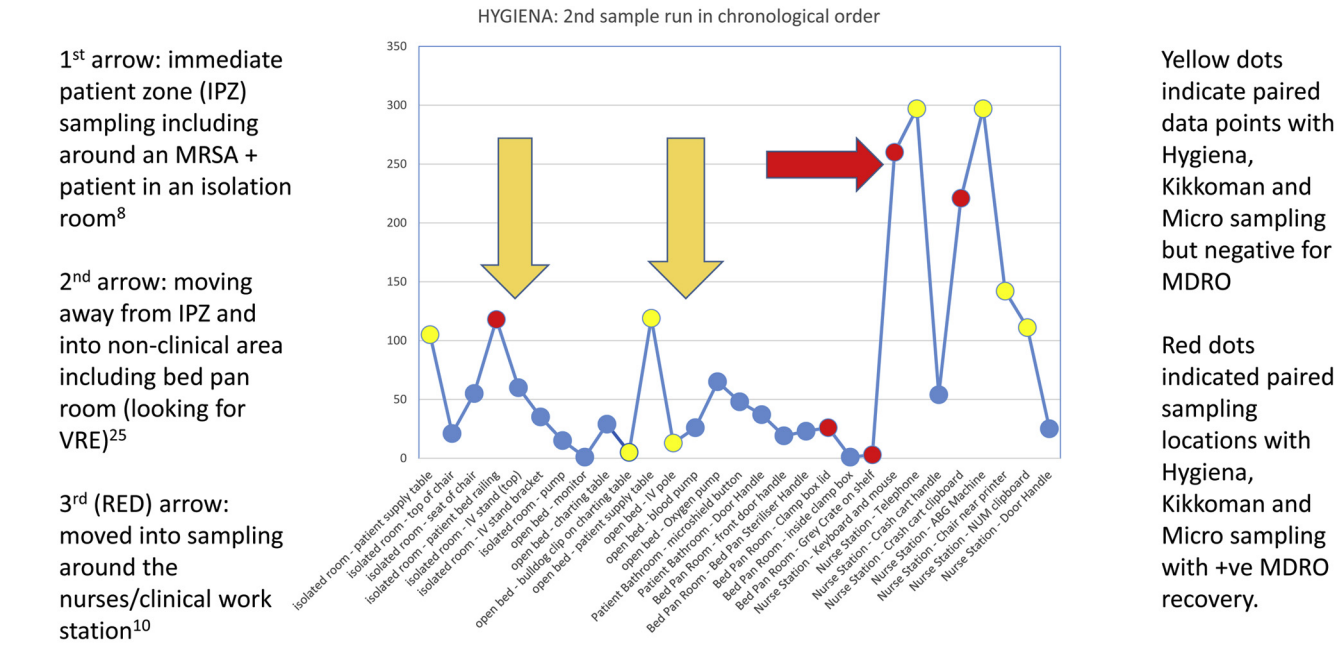

Fig 1. Second sampling run, all sample locations using Hygiena ATP testing.

swabs and both brands of ATP tests, identified MDROs at 12 of 36 sampling locations (MDRO recovery rate of 33.3%; n = 36).

Using this abridged approach, the first sampling run using only microbiology swabs to locate MDROs (4.3%) was compared with the combined matched dataset from the second and third sampling runs (36%). The MDRO success rate with and without the use of ATP to guide sampling locations was tested and found to be significantly associated using both the χ^2 test (with Yates correction; $P < .025$) and Fisher's exact test ($P = .01$).

The data from the first sampling run and matched data from the second and third sampling runs are summarized in Table 3. As shown in Table 3, the Hygiena device has a lower reading range (in

Table 2
Confirmed locations of MDRO

HTO	Hygiena	Kikkoman	MDRO
Patient folder on patient notes bench	No reading	No reading	MRSA
Isolation room, patient bed railing	118	639	MRSA
Bedpan room, clamp box lid	26	2,671	VRE
Bedpan room, gray crate on shelf	3	786	VRE
Clinical station, keyboard and mouse	260	2,436	VRE
Clinical station, crash cart clipboard	221	1,125	VRE and ESBL (*Enterobacter cloacae*)
Clinical station, computer keyboard, space bar	19	443	*Enterococcus faecalis*
Clinical station, crash cart clipboard, rear side bottom left corner	24	942	*Acinetobacter baumannii*
Clinical station, chair height adjustment handle, front	27	2,833	*Pantoea agglomerans*
Clinical station, crash cart clipboard, front side, top left corner	32	378	MRSA
Clinical station, chair, top of back rest, center front (fabric)	77	1,321	*Acinetobacter pitti*
Clinical station, chair, side of seat, center (fabric)	106	483	*Acinetobacter pitti*
Clinical station, chair, side of seat, center (fabric)	134	425	*Enterococcus faecium*

ESBL, extended-spectrum beta-lactamase; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *VRE*, vancomycin-resistant enterococci.

Table 3
Data summary from the first sample run and matched data from the second and third sample runs

	Samples	MDRO identified	MDRO negative
First run, n (%)	23	1 (4.3%)	22 (95.7%)
Second and third runs, n (%)	36	12 (33.3%)	24 (66.7%)
Hygiena range for MDRO	36	3-260 RLU (average, 87.3 RLU; median, 54.5 RLU)	5-297 (average, 69.6 RLU; median, 31.0 RLU)
Kikkoman range for MDRO	36	378-2,833 RLU (average, 1,206.8 RLU; median, 864 RLU)	106-36,452 RLU (average, 2,449.2 RLU; median, 845.5 RLU)

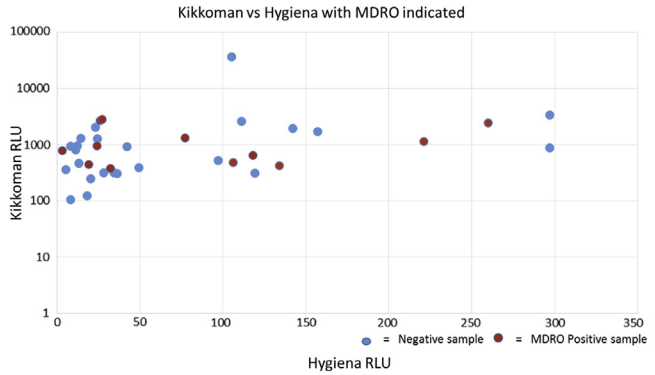

Fig 2. Data plotted for MDRO and ATP readings.

RLU) than the Kikkoman device. In this study, the Hygiena device recorded readings ranging from 3 to 260 RLU for MDRO-positive locations and from 5 to 297 RLU for MDRO-negative locations. Compared with the Kikkoman device, both datasets for the Hygiena device showed lower average and median results. The average and median results were quite well separated, with the average higher than the median. The median results for the Hygiena device were gigher for the MDRO-positive locations.

Application of the χ^2 test demonstrated that the Hygiena device was incapable of indicating MDRO locations when a cutoff of 15 RLU was applied ($P > .15$ with 1 df). The cutoff value of 15 RLU was determined using a receiver operating curve sensitivity analysis.

The results for the Kikkoman ATP device showed readings ranging from 378 to 2,833 RLU for MDRO-positive locations and from 106 to 36,452 RLU for MDRO-negative locations. The Kikkoman data also showed a significant separation between the average and median values.

Our χ^2 analysis failed to demonstrate that the Kikkoman ATP device was capable of indicating MDRO locations at a cutoff of 350 RLU ($P > .15$ with 1 df). This 350 RLU cutoff was determined using a receiver operating curve sensitivity analysis.

Data for the Kikkoman and Hygiena devices are plotted together in Fig 2. There was no correlation between the 2 sets of readings ($R^2 = 0.0144$).

DISCUSSION

In this pilot study, it would be easy to overstate the significance of the results, owing to a range of potential methodological flaws. These concerns include the selection method used for sampling of HTOs, the introduction of a more experienced operator with ATP sampling in the second sampling run, the variability of readings with the portable ATP devices as a potential confounder, the aggressive swabbing technique used to locate MDROs and potential biofilm-related organisms, and lack of assessment of total heterotrophs in the microbial sampling. The statistical analysis was complicated, because a fully matched dataset could not be obtained from the second sampling run.

Nonetheless, the ATP testing served as a relative indicator of surface cleanliness, and the timely results guided the microbial sampling, which was otherwise weakened by the delay in obtaining results of microbial sampling in isolation.[24] As indicated in Fig 1, the timeliness provided by the ATP testing contributed to the significantly improved sampling outcomes when searching for MDROs on surfaces within and around the ICU ($P < .025$, χ^2 test with 1 df, and $P = .01$, Fisher's exact test). The results were significant even with all microbial samples (including 3 nonpaired samples) included ($P < .05$, χ^2 test with 1 df; $P = .021$, Fisher's exact text).

The purpose of cleaning is to remove soils including bacteria and other biological materials; however, cleaning has been described as a weak science, with poor reliability.[29] Part of the challenge in improving cleaning performance is to enable better science that allows staff to monitor and identify where the microbes of concern are located, and then whether or not the cleaning process has been efficacious.[30] The use of rapid ATP testing for the purpose of assessing HTO cleanliness provides practical assistance for improving the discovery rate for MDRO locations above that using literature or visual inspection alone as a guide to sampling locations.

The initial sampling of HTO was focused around the immediate patient zone and the clinical treatment area with reference to

recognized HTOs. When ATP testing was included, the higher RLU readings from the 2 ATP devices suggested the need for intensive investigation of the staff clinical area, in which all clinical staff were frequently moving to and fro, with both ATP devices and environmental swabbing (Fig 1). The majority of MDRO-positive locations were identified in this area.

In this pilot study, insufficient data were available to test the significance of the differences in RLU readings between HTOs in the immediate patient zone and those in other areas within the ICU. In the clinical workstation, the focus of sampling was on HTO sites that were later found to be uncovered by any cleaning protocol or cleaning responsibility. The list of contaminated HTOs and the range of MDROs recovered is provided in Table 2.

Using the ATP devices significantly improved the sampling approach. A cutoff of 350 RLU could reasonably be applied to the Kikkoman readings, even though the threshold was not significantly associated with the likelihood of finding an MDRO. A cutoff of 15 RLU when using the Hygiena device might be possible for all but 1 of the MDRO locations indicated with >15 RLU, which is a recognized standard for school cleaning.[31]

Our results underscore the danger of applying a single 100 RLU cutoff without first testing the appropriate cutoff for a particular ATP device.[32] With single point sampling results from ATP testing, there is a 20% chance of reading imprecision by a factor of 2.[28] This variability is also noticeable in the noncorrelation between the 2 devices shown in Fig 2 ($R^2 = 0.0144$).

As indicated in Table 3, the RLU readings from the 2 ATP devices were 1 order of magnitude different at the average levels and more than 1 order of magnitude different at the median levels. This finding also highlights the danger of using average readings between different brands of ATP devices.[20] The reason for the difference in relative sensitivity between the 2 branded ATP tests was not determined, but may be related to variability in swab material, lysis systems, or light detection, or even in broad reactivity of the underlying biochemistry of the enzymes used in the swabs.

In this pilot study, we found that many of the HTOs from which MDROs were recovered were not items included in cleaning protocols.[10] Such items as telephones, computer keyboards, and chairs were sampled in between use by clinical staff. Other items (eg, the crash cart clipboard) were not used during the sampling runs, but were observed to be handled on several occasions to access nearby drawers, cupboards, and, on one occasion, a handwashing sink.

Hand hygiene compliance was not audited during this pilot study, but is typically reported as adequate.[33] Clinical staff were observed to use items in the clinical area both with and without gloved hands. Terminal cleaning by cleaning staff using disposable chlorine wipes inside vacated patient zones was observed during the sampling. No cleaning of the clinical workstation was observed during any of the 3 sampling runs.

In conclusion, the findings of this study suggest the need to review the hygiene standards adopted in the clinical workspace, away from the immediate patient zones in busy ICUs, and indicate that ATP testing may help identify HTOs with less than optimal cleanliness. Better monitoring of cleaning may improve the surface hygiene outcomes from effective cleaning protocols applied to all HTOs within and around clinical work areas inside ICUs.[24] This pilot study was part of a wider project, and other studies of the various components required for hygiene improvement within health care settings are currently underway.

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Section Three

Appendixes

Appendix A

Other papers published as part of the PhD study

Appendix B

Posters presented as part of the PhD study

Appendix A

Other papers published as part of the PhD study

A1 **Letter to the editor: The importance of stakeholder consultation for infection control guidelines: Healthcare Infection: 2009:14:9**

A2 **Letter to the editor: Flawed recommendations on surface hygiene within the existing Interim Influenza Pandemic National Infection Control Guidelines: Healthcare Infection: 2009:14:177-179**

A3 **Letter to the editor and reply:**

Letter: Whiteley GS et al. Sampling plans for the use of rapid adenosine triphosphate (ATP) monitoring must overcome variability or suffer statistical invalidity: Infect Control Hosp Epidemiol: 2015:36:236-237

Reply to Whiteley: Infect Control Hosp Epidemiol: 2015:36:237-238

A4 **Letter to the editor and reply:**

Letter: Roady L: The role of ATP luminometers in infection control: Infect Control Hosp Epidemiol: 2015:36:1367

Reply to Roady: Infect Control Hosp Epidemiol: 2015:36:1368

Appendix A1

Letter to the editor

The importance of stakeholder consultation for infection control guidelines

Letter to the editor

The importance of stakeholder consultation for infection control guidelines

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We write regarding the Australian Infection Control Guidelines (AICG), last published in 2004 and now locatable on the Australian Government website at http://www.health.gov.au/internet/main/publishing.nsf/Content/icg-guidelines-index.htm

When the AICG were first published the then process was that there were several working drafts circulated to all of the stakeholders for consultation. When one visits the website version of the AICG, the face sheet is the same, the publication date is indicated as 2004, and even the index is the same.

However, if one has a copy of the original printed version, once you get into the body of the document you will find significant differences. The website indicates that some amendments have been made in December 2007, however, without a comparison copy the extent and nature of these changes is not obvious. We highlight three unacceptable changes in the internet version.

First, is the recommendation of the use of anionic detergents in the chapter on prions and related diseases (Chapter 31). In the original there were only two references to anionic detergents for cleaning. However, an investigation of the revised chapter on prions repeatedly and widely indicates the use of anionic detergents for pre-cleaning of potentially contaminated instruments. The recommendations are unreferenced and contradict the literature, which is confused at best about anionic detergents as a mandated step. We cannot determine where the original reference arose.

Second, the revision of a single section has introduced some major contradictions. For example, section 31.3.3 of the current (2007) version states "instruments potentially contaminated with higher-infectivity tissue should be immersed in a dedicated container in sterile water until they are reprocessed", which contradicts the discussion point in an earlier section (16.3.4), which states "Instruments potentially contaminated with CJD agents should be kept immersed in a dedicated container in an anionic detergent solution, at ambient temperature, until they are manually cleaned and reprocessed using the methods shown in Table 31.9".

Third, is the recommendation in the same chapter for the use of ultrasonic cleaning machines for cleaning potentially contaminated instruments. This represents an infection risk due to the creation of aerosolised particles above the liquid phase. No safety instructions are given and the recommendation is not referenced (and is in fact in contradiction with advice given earlier in section 16.3.4).

In summary, the new amendments have not been subject to widespread consultation. This is in contravention of official government policy and the Council of Australian Government (COAG) Principles. The errors contained in the document might increase risks for health care workers. This is a good example of why consultation among all of the stakeholders is always a good plan for a guidelines document of this nature.

Conflicts of interest

Whiteley Corporation manufacturer a range of cleaning, decontaminating and disinfecting products for reuseable medical devices and hospital surfaces.

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Appendix A2

Letter to the editor

Flawed recommendations on surface hygiene within the existing Interim Influenza Pandemic National Infection Control Guidelines

Flawed recommendations on surface hygiene within the existing Interim Influenza Pandemic National Infection Control Guidelines

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Abstract

The Interim Infection Control Guidelines for Pandemic Influenza in Healthcare and Community Settings, published by the Australian Commonwealth Government in 2006, have been reviewed and found to contain recommendations that, if they were made by a private organisation, would be potentially illegal under the Therapeutic Goods Act 1989, and also raise some concern in relation to occupational health and safety. The guidelines also fail to recommend the use of disinfecting products with specific claims relating to influenza virus that have been approved by the Therapeutic Goods Agency.

In 1996, the Australian Government through the gazetting of Therapeutic Goods Order Number 54, 1996 (as amended), introduced a range of controls over the disinfectant market in Australia. During the following year, all states of the Commonwealth formally ceded their powers in this area to the Australian Government. The Therapeutic Goods Administration (TGA) administers the regulatory scheme, which was augmented in 2002 with the Medical Devices Regulations (2002) to cover not just the higher risk products under a uniform international scheme, but also to bring under control even the cleaning products intended for use within healthcare. The TGA, through a range of instruments, also controls the label claims of all products in this category, and for any of the products intended for use exclusively within healthcare, this is a pre-market scheme. In practice, this means that any therapeutic claims (such as efficacy in disinfection of, for example, influenza virus) are required to be approved by the TGA, and that in order to gain TGA approval, the manufacturer is required to submit fully validated test data for review. It is therefore particularly strange that the Australian Guidelines for Infection Control within the context of an influenza pandemic[1] do not even mention the TGA as the regulator of therapeutic goods. The recommendations contained within these guidelines for surface disinfection focus generically on alcohol and chlorine as active materials. There are four areas of concern over the recommendations as they are currently framed. The affirmation of these guidelines by distinguished authors[2] lends weight to the opinions expressed within the guidelines, but fails to address the legal requirements of the Therapeutic Goods Act or allow for a hierarchy of control so important in managing risk, and particularly risk in occupational safety.[3]

First, there is the equity problem of unqualified recommendation of products by brand name within the official interim guidelines where the opportunity of consideration of legal alternatives is not provided. The products named are chlorine-based disinfectants without any specific approved claims (e.g. for killing of the influenza virus on surfaces). The most galling situation is the specific recommendations superimposed, wherein information is provided by the guidelines which if repeated by the companies involved would be strictly illegal under the Therapeutic Good Act 1989. These guidelines as they stand are very good for the company named, but deny equity to all other companies, even when some other companies have products registered and accepted to kill influenza virus, information that the named products do not have on their official labels.

Second, while the strengths recommended for disinfection (1000 ppm or 0.1%) would not be classifiable as hazardous under the Australian Occupational Health and Safety regulations,[4] such solutions are not available off the shelf. In the case of liquid chlorine, the actual disinfectant solution will be prepared by dilution of a significantly higher concentration solution. Such solutions are highly corrosive and classifiable as both a hazardous substance and a dangerous good under Australian Occupational Health and Safety regulations. Sodium hypochlorite solutions are also notoriously unstable with respect to loss of active chlorine, and therefore a means of analysing for active chlorine is essential to ensure a correct working dilution.

Solid chlorine (sodium dichloroisocyanurate), although more stable than liquid chlorine, is also a hazardous substance (irritating

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to skin and eyes, and the respiratory system). It is also a powerful oxidising agent (classified under the Australian Dangerous Goods Code as a class 5.1 oxidising agent). There is therefore a workplace requirement[5] to segregate this material from certain other dangerous goods (e.g. class 3 flammable liquids such as alcohol). By contrast, many hospital grade disinfectants with TGA approved claims relating to influenza are not classifiable as hazardous substances or dangerous goods.

Third, the recommendation for use only of chlorine-based disinfectant, at 1000-times the strength of what is used in swimming pools, is completely impractical. During a pandemic, the need for surface disinfection is not limited to clinical areas. For example, a patient waiting room will be a mixed environment with fabrics, carpet, magazines, chairs and other objects that may become contaminated by droplets or fomites from a coughing and sneezing viremic patient. The use of chlorine-based disinfectants may also affect safety measures such as the fire resistance of upholstery.[6] Clearly, the inexpensive option of a chlorine-based solution will be likely to lead to damage that far outweighs the initial cost of purchase. This is so unnecessary when there are products registered to kill influenza virus on surfaces, which are not corrosive, are non-staining and are safe to use when used as directed on the approved label.

Fourth, the unpleasantness and impracticality associated with use of chlorine products at this strength will likely also lead to incorrect, insufficient or inappropriate non-use. This elevates the recommendations to the level of the absurd. It is human nature that unpleasant tasks are avoided. The problems with hand washing compliance are a case in point. Widespread non-compliance with the recommendations will almost certainly lead to the psychological phenomenon known as 'normalised deviance' and in fact raise the risk of transmission via contaminated inanimate surfaces.

Readers should be aware that this is not at all a fanciful problem, as evidenced by the findings on the last two major pandemics that affected Australians. (i) SARS, involving a coronavirus, was spread and its virulence increased via inanimate surface spread.[7,8] (ii) The 2007 outbreak of equine influenza occurred primarily due to a breakdown in surface disinfection within the quarantine process, which saw the virus escape quarantine via inanimate objects taken out of quarantine by humans and then infect other horses.[9] The Honorable Ian Callinan, who led the Commission of Inquiry into the Equine Influenza outbreak, said about the outbreak:

> 'I find that the most likely way that the virus entered the general horse population is by its escape from infected horses at Eastern Creek Quarantine Station on contaminated person or persons or equipment leaving the Quarantine Station and coming into contact with a horse.'[9]

The current Interim Infection Control Guidelines for Pandemic Influenza in Healthcare and Community Settings (2006) make the point that the human influenza virus does remain viable on surfaces for up to 48 h.

The problems that have arisen in the guidelines could have easily been avoided through a consultation process in accordance with the accepted Council of Australian Governments recommendations. A consultation process with the stakeholders involved in this sector would have pointed the authors back to legislative process and included the correct references to the regulator. The corrections are quite simple.

Recommendations

(1) Remove all reference to company brand names.
(2) Recommend use of only TGA approved hospital grade disinfectants with label claims noted against influenza virus on surfaces.
(3) Refer inquirers with questions on specific products to the online portal for the Australian Register of Therapeutic Goods for information regarding the general approval status of products being recommended by category.

At the present time, the Australian Infection Control Guidelines are undergoing a major revision by the Australian Commission on Safety and Quality in Health Care, and this revision offers an opportunity to bring the Australian Infection Control Guidelines in line with not only global best practice, but with Australian Therapeutic Goods legislation. In this respect, it is perhaps advisable to include both regulators and industry representatives alongside clinicians while drafting such guidelines. It is also highly recommended that all guidelines should be reviewed by the relevant governmental agencies for compliance with existing and upcoming legislation.

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Appendix A3

Letter to the editor and reply

Appendix A3.1

Letter: Whiteley GS et al.

Sampling plans for the use of rapid adenosine triphosphate (ATP) monitoring must overcome variability or suffer statistical invalidity

Appendix A3.2

Reply to Whiteley

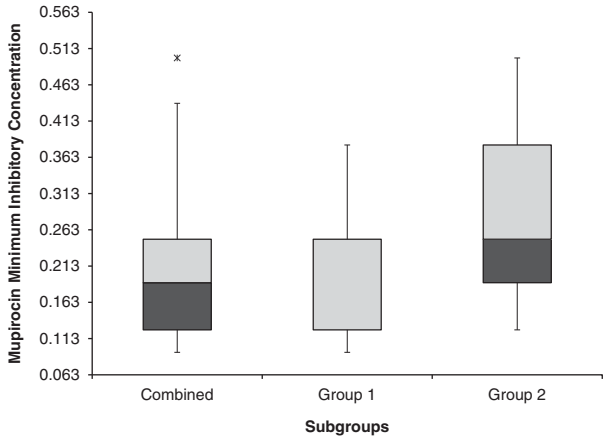

FIGURE 1. Box-and-whisker chart of mupirocin-susceptible, methicillin-resistant *S. aureus* isolates. Left to right: combined groups 1 and 2; group 1, tissue isolates; group 2, nares isolates.

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Sampling Plans for Use of Rapid Adenosine Triphosphate (ATP) Monitoring Must Overcome Variability or Suffer Statistical Invalidity

Reply to Visrodia

To the Editor—We write with respect to the article by Visrodia et al.[1] on using a commercial rapid adenosine triphosphate (ATP) device for validation of cleaning of flexible gastroendoscopes. The importance of timeliness in quality assurance testing in this device area is critical owing to the time pressures on the use of the gastroendoscopes by clinical staff involved in patient care. The work is a useful additional contribution to this growing field of use for ATP devices.[2]

Nonetheless, we highlight concerns with 2 aspects of the method adopted within the work by Visrodia et al.[1] First, this work, like earlier references, utilizes only a single brand of rapid ATP device with acknowledged manufacturer support. The recommendations on "validated" relative light units (RLU) are entirely device specific and exclude other commercial devices. And, whilst the ATP/RLU readings in Visrodia et al.[1] may seem dramatic (some > 100,000 RLU), the work lacks evaluation of microbial presence that could anchor the study against a quantitated standard.[3]

Second, the work does not address any of the major published criticisms of the use of ATP systems as they are currently configured. Several authors have commented on the dangers of overstating the usefulness of these commercial ATP devices, the risks of alternative sources of ATP, the lack of correlation with specific pathogens of concern, the amount of ATP present within any particular cells or bacterial species, and the measurement variability that undermines statistical measures applied to the research.[4–7]

In this regard, and of specific concern in terms of method in Visrodia et al.,[1] is the way that ATP measurements and samples were obtained—for example, samples from the brush and flush sampling were divided into only 2 parts, with one part apparently used for a single ATP test and the other part tested for protein residues. The entire sample set of

ATP testing appears to be without duplicates or preferably triplicate testing. Reliance by Visrodia et al.[1] upon the sample means of groups of singular ATP readings is undermined by the knowledge of variability where the standard deviation can be as high as 40% of the data mean for the individual brand of device used.[8] The authors themselves note the risk of singular testing in the body of the discussion: "to sample more than one… and to use more than 1 rapid indicator," but we wonder how the statistical assumptions hold valid without multiple (replicate) samples taken for the ATP testing.

We also note 2 problems with the scaling of all commercial ATP devices. First, the scale of RLU is completely relative and cannot be used interoperatively between differently branded devices.[2,3] Second, the variability for each of the brands is so high that without a sampling approach that accounts for multiple samples at any one point, the ability of the scientists involved to meaningfully apply statistical methods renders the article subject to first principle flaws.[9] Reporting the RLU readings on a log scale is not the same as taking multiple samples, identifying the median value, and then log plotting the data. Perhaps this was done, but it remains unclear within the text.

We feel obliged to inform those who may be reliant upon the work to take care in not applying the work using one brand of ATP device to another brand of ATP device, as noted in the commentary by Petersen.[10] Likewise, we caution against relying on the statistical positioning in the field use of ATP without an appropriately constructed sampling plan to account for inherent variability. This overlay of concern will continue to apply until all ATP device manufacturers can agree to a commonly applicable scale that minimizes the impact of variability, no matter what the assignation given to the replacement reading scale.

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Reply to Whiteley et al

To the Editor—We appreciate the commentary by Whiteley et al[1] on our study in which several rapid indicators were used to detect residual contamination in gastrointestinal endoscopes following manual cleaning.[2] The authors raise several concerns about an adenosine triphosphate (ATP) measuring device used in our study, including our use of a single commercially available ATP device, our reliance on only 1 ATP test per component sampled, possible variability in ATP results, and the inability of ATP monitors to identify specific microbes or quantify colony counts.[1] Indeed, rapid indicator testing in endoscope reprocessing is a relatively new arena, and more research is undoubtedly needed to evaluate the utility of various devices and determine the association between residual organic debris, viable microbes, and patient outcomes.

Our study was a small pilot project designed to evaluate materials and methods that could be used to assess endoscope cleaning effectiveness. At that time, we sought to determine whether the recommended practice of visual inspection was an adequate standard for verifying whether manual cleaning had

sufficiently removed residual contamination prior to exposing endoscopes to high-level disinfection.[3,4] In addition to inspecting each component and the sampling materials for visually apparent evidence of residual contamination, our team conducted rapid indicator tests for blood, protein, and ATP. Multiple types of rapid indicators were used in order to assess various approaches for monitoring cleaning effectiveness and to compare their results. In summary, we found[2] that endoscopes with and without visually apparent debris had levels of blood, protein, and ATP exceeding previously validated benchmarks.[5,6] Although high ATP levels may indicate the presence of viable microbes,[7,8] such results could also reflect the presence of blood or other types of cells.[9] Indeed, we found ATP levels were quite high in every sample that also tested positive for blood.

As noted by Whiteley et al[1] and acknowledged in the limitations section of our article, we did not include the performance of microbial cultures, because the goal was to evaluate multiple rapid indicators and sample collection methods. Furthermore, the value of conducting microbial cultures prior to high-level disinfection seems limited. In a subsequent study conducted by our team (as yet unpublished) we used microbial cultures as one of the indicators of endoscope reprocessing effectiveness.

The main goal of our study was to identify user-friendly materials and methods that could be used to evaluate manual cleaning effectiveness in the clinical setting. The chosen ATP monitoring system provided a numerical result reflecting the amount of ATP present. We found this to be superior to monitoring systems that measure residual protein or blood, which require users to interpret color changes on swabs or dipsticks.

Given the imperative for cost containment and to improve efficiencies on the front lines, we believe it would not be desirable to perform duplicate or triplicate testing as suggested by Whiteley et al.[1] Their concern about variability within and between ATP measuring devices deserves additional study. However, we found that post–manual cleaning ATP and protein levels far exceeded benchmarks for manually cleaned endoscopes and perhaps are less likely to be affected by the degree of variability cited.

Quality assurance in endoscope reprocessing is needed, and rapid indicator testing is an area of growing interest and understanding. ATP testing offers potential, but given its relatively recent application to this field, additional research is necessary to better define its role.

ACKNOWLEDGMENTS

We thank Evan Doyle, BS, and Otis Heymann, BA, for their editorial assistance.

Financial support. C.L.O. reports that she is employed by Ofstead & Associates, which has received research funding and speaking honoraria related to infection prevention from 3M Company, Medivators, Boston Scientific Corporation, invendo medical, Steris, Johnson & Johnson, and Ecolab. H.P.W. reports that he is employed by Ofstead & Associates.

Potential conflicts of interest. 3M Company provided funding and materials to Ofstead & Associates for the study discussed in the Commentary (to which this letter replies). 3M Company did not have access to any study data and was not involved in the preparation of this letter. Additional research support was provided by Mayo Clinic and Ofstead & Associates. Neither the physicians at Mayo Clinic (K.H.V., P.K.T.) or University of North Carolina (T.H.B) nor their departments received monetary compensation for participating in the study.

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Appendix A4

Letter to the editor and reply

Appendix A4.1
Letter: L Roady
The role of ATP luminometers in infection control

Appendix A4.2
Reply to Roady

LETTERS TO THE EDITOR

The Role of ATP Luminometers in Infection Control

To the Editor—I would like to caution readers on the interpretation of the article by Whitely et al[1] in this issue. This study, like many before, makes the mistake of assessing ATP luminometers as bacterial detection systems. Studies have demonstrated,[2] and responsible manufacturers will confirm, that there is not a 100% direct correlation between ATP and plate counts, so evaluating an ATP system's performance based on bacterial detection is an impractical assessment of the tool. For hospitals seeking to monitor microbial cleanliness, the necessary testing method is conventional microbiology plating. ATP testing is not a substitute for microbiology testing, and responsible ATP manufacturers do not make that claim. Furthermore, the author makes a hasty judgment on the value of ATP systems: "The original suggestion to use rapid adenosine triphosphate (ATP) bioluminometers to monitor surface hygiene has yet to see practical fulfillment among healthcare infection control and prevention practitioners."

In reply, I direct readers to the abundant published evidence to the contrary.[3–8] ATP testing is designed to demonstrate whether cleaning regiments are working correctly and whether cleaning agents and techniques are working properly to remove biological contaminants such as blood, protein tissues, skin cells, etc., which can facilitate microbial growth. ATP testing plays a key role in training, process improvement, and ongoing monitoring of overall hospital cleanliness. There is undeniable and plentiful evidence that monitoring cleaning with an objective tool like ATP testing improves cleaning thoroughness, improves staff training, and optimizes cleaning regiments. In fact, ATP testing has been proposed as an acceptable method by the Centers for Disease Control and Prevention (CDC) in the United States and accepted as a national standard by Danish government. The benefits of ATP are also recognized in the UK *National Health Service Healthcare Cleaning Manual* (2009).

ATP testing has proven effective as an intervention tool over and over. In comparison to other proposed methods such as fluorescent gel black light or visual inspection, ATP testing is the best available option to monitor cleanliness other than microorganism testing, which is impractical for the determination of room cleanliness.[9–11] ATP testing systems are the best available option for improving cleaning regiments, training employees, demonstrating effectiveness of cleaning agents, and improving hygiene in the environment with the end goal of reducing the spread of infection. ATP systems play a very important and valuable role in overall efforts to improve cleaning. Considering the purpose of the ATP systems and additional studies cited, there is overwhelming evidence that ATP testing is a very valuable aspect of cleaning monitoring and improvement within healthcare industry.

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Potential conflict of interest. Lauren Roady, MBA, is Marketing Manager for Hygiena, one of the ATP technology brands referenced in Whiteley, et al.[1]

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Reply to Roady

To the Editor—We welcome the engagement and comments of Ms. Roady in response to our research article.[1] We agree that the use of rapid ATP testing has a growing body of published support. However, the lack of common acceptance for rapid ATP testing at this point in time is well expressed in the EPIC 3 Guidelines (2014) from an expert committee in the United Kingdom.[2]

Unfortunately, Ms Roady somewhat misses the point of our paper. In our study, we did not attempt to equate rapid ATP testing with detection of bacterial contamination. We showed that the variability that occurs when measuring responses to controlled quantitated microbial cultures is the same variability that occurs when controlled concentrations of pure ATP solutions are measured. The issue is therefore not correlation with detection but data variability.

This variability is undetectable to ATP device users and applies to all sources of detected ATP. The ATP variability problem (ie, imprecision in results) that we have outlined in our most recent paper is common to each of the ATP device brands we tested. This finding does have implications for sampling methodology and analysis.[3]

We set out to validate several branded ATP devices using a standardized approach and focusing on precision and accuracy.[4] The first issue we encountered was uncontrolled variability and the lack of precision at any testing point. The issue of accuracy is problematic because the scale of relative light units (RLU) is neither universally standardized nor standardized among ATP device suppliers.

We welcome the engagement with the industry. We would like to see better quality of results for ATP testing devices, including testing for precision and the development of a common measurement scale. There remains a tremendous upside for ATP use once these issues are resolved.

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Infection Control Implications of Protracted Lengths of Stay With Noninfluenza Viral Influenza-Like Illnesses in Hospitalized Adults During the 2015 Influenza A (H_3N_2) Epidemic

To the Editor—Infection control (IC) precautions for hospitalized adults with influenza consist of standard, contact, and droplet precautions with single rooms recommended or with patients cohorted, but guidelines for viral influenza-like illnesses (ILIs) are not standardized.[1] During the January 2015 influenza A (H_3N_2) epidemic in our location, the high volume of patients with ILIs became problematic, creating a major strain on bed availability.

In January 2015, a total of 54 adults were admitted with influenza A (H_3N_2) and 37 adults were admitted with viral ILIs diagnosed by multiplex polymerase chain reaction (PCR) assay of nasopharyngeal swab samples. Of the 54 influenza case patients, 53 (98%) had influenza A (H_3N_2) and 1 (2%) had influenza B. One patient had a dual-positive rapid influenza diagnostic test for influenza A and influenza B, but PCR testing was positive for influenza A (H_3N_2).[2–6]

Of the 37 adults with viral ILIs, 16 (43%) had respiratory syncytial virus (RSV), 10 (27%) had rhinovirus/enterovirus (R/E), 5 (14%) had human parainfluenza virus type 3 (HPIV-3), 4 (11%) had human metapneumovirus (hMPV), and 2 (5%) had coronavirus (Table 1). Elderly patients, more commonly admitted for viral ILIs, had longer LOS for viral ILIs than for influenza. RSV patients were older (mean age, 83 years), with LOS similar to that of influenza patients. Importantly, HPIV-3 patients had the longest LOS of any viral ILI (mean, 19 days) and were more seriously ill, with 1 death due to HPIV-3 pneumonia. Two patients had co-colonization

Appendix B

Posters presented as part of the PhD study

B1 **The Importance of Practical Zero in cleaning performance indicators: IFIC Conference 2011**

B2 **Validation of cleaning indicators for healthcare surfaces: IFIC Conference 2011**

B3 **The problem of rapid ATP systems many be scaling using Relative Light Units (RLU): HIS Conference 2014**

B4 **Evidence based cleaning and cleaning validation using FM and ATP systems: HIS Conference 2014**

B5 **A new method for practical quality control in monitoring the cleaning of healthcare surfaces: ASID Conference 2015**

The Importance of Practical Zero in Cleaning Performance Indicators

Greg S Whiteley[a,b]; Chris Derry[a]

a = University of Western Sydney; b = Whiteley Corporation

University of Western Sydney
Bringing knowledge to life

Introduction

Accurate assessment of cleaning effectiveness is critical to reducing the incidence of multiple drug resistant organisms (MDRO) in the healthcare setting (Dancer 2009). The Australian requirement (NHMRC 2010) for direct visual inspection (DVI) of surfaces as primary monitoring strategy is echoed in standards and guidelines elsewhere. This approach is subjective and inaccurate and liable yield an unacceptable number of false negative results for surface contamination (Griffith et al. 2000). New quantitative monitoring methods are emerging but these present new range of challenges, one of which relates to the imprecision of the point at which a surface can be considered “clean”. Microbiological tests can offer a definitive cut-off, such as the presence or absence of pathogens, but delay in receiving results remains a problem. While results of innovative tests such as ATP-bioluminescence monitoring are immediate, interpretation of results still requires research in terms of the relationship of results to actual risk in terms of safe exposure threshold to determine a “practical zero” to guide surface cleaning practitioners. This requires the development of a risk assessment methodology, towards which some basic ideas are presented in this poster.

Methods

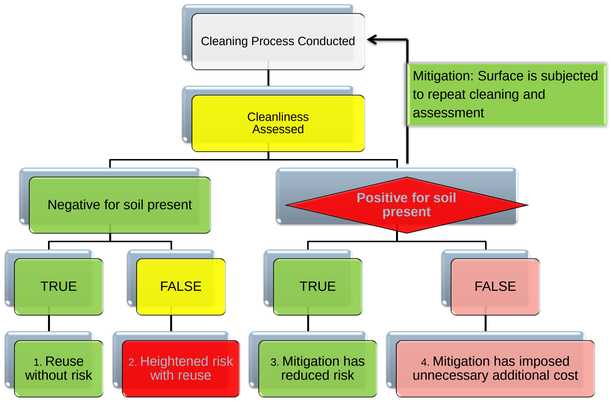
1. A literature review was carried out and a model developed to represent steps in the cleaning and monitoring process with risk-related outcomes. The “false negative” was then identified as the critical outcome in terms of human exposure risk to contaminated or inadequately cleaned surfaces.

2. The Australian guidelines and standards relating to cleaning in the healthcare setting were reviewed and the common practice of relying on DVI determine practical zero identified (AG:DHA 2004; NHMRC 2010; NSW Health 2007; VG:DHHS 2011; SA/SNZ 2003).

3. DAZO(o) and (e) (original and Ecolab) chemiluminescent marker was sourced. Each type was subjected to drying and naked-eye visibility measurements as well as definitional description under UV light, in terms of expected practice (Carling et al. 2006; Carling et al, 2008). Results were recorded and photographed.

4. The performance of three commercial brands of hand-held luminometer (3M’s Biotrace, Kikkoman’s Lumiscan and Hygiena’s Ultrasnap) were assessed against LC-MS determined ATP curves in terms of recommended thresholds (Lewis et al. 2008; Andersen 2009; Boyce et al. 2009; Mulvey et al. 2011) and using recommended procedures (ISO 13485:2008, VG:DHHS 2005).

Results



Outcome-driven risk assessment model

Preliminary intervention

Feedback for mitigation Assessment

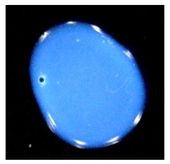
Basic assessment outcome

Four outcome conditions

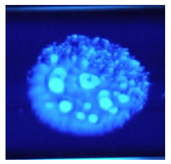
Four outcome implications

Cleaning indicator tools for validation purposes:

DAZO (original)



DAZO (E -version)



Discussion

Following preliminary validation work on traditional and new testing procedures, it was found that a model based on the detection of false negatives had capacity to accommodate all monitoring types as the starting point for more complex risk modeling relating to the determination of safe exposure thresholds for residual contamination on surfaces. To facilitate use of such a model, however, other risk factors need to be identified and accommodated if effective and early feedback is to be given to staff in the interests of risk mitigation. Such risk factors include level of MDRO present, presence of other material (protective residue or biofilm), frequency of exposure (high touch objects [HTOs] being particularly relevant), nature of surface (texture and composition) and patient immune status. To guide cleaning the concept of acceptable risk needs to be expressed as a definitive “practical zero” result which can guide the cleaning process.

During validation it was found that DAZO chemiluminescent markers dried effectively although the DAZO(o) had greater mass, dried slower and showed some hydroscopicity in comparison to DAZO(e). DAZO(o) also had a smaller footprint per volume with better-defined boundaries (see above photographs). There was some visibility of deposited spots which is an undesirable quality in tests required to develop a practical zero relating to traditional cleaning practice which uses DVI as an integral part of the process.

Validation testing of ATP-bioluminescence monitors is underway although early results have suggested some variability of individual meter performance against ATP standard curves, the generation of some false negatives when instruments are required to operate at a high level of resolution close to the critical measurement range, and drop-off in measurement accuracy as reagents approach the end of their shelf-life. The ability of such meters to measure to very low levels of ATP, however, offers possibilities for their role in identifying a practical zero.

Conclusion

This poster suggests a risk assessment framework which might be used as a common vehicle for determining safe exposure thresholds in the derivation of practical zero in assessment of cleaning relating to a range of HTOs in different healthcare settings.

While microbiological assessment can provide a benchmark for retrospective surface monitoring, proactive monitoring in terms of the restrictive time frame applicable to infection and cleaning cycles requires other methodologies.

For many years, reliance has been placed on direct visual inspection which, although immediate, has shown to be inadequate in terms of accuracy. While other proactive alternatives to visual inspection are available, research is needed to contextualise the relative advantages and disadvantages of each within a broad risk management framework, and here concepts such as safe threshold and practical zero require further investigation.

The cut off point for a realizable practical zero should be at or below the point at which the possibility of a false negative outcome occurs.

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Validation of Cleaning Indicators for Healthcare Surfaces

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University of Western Sydney
Bringing knowledge to life

Abstract

Technology including ATP measurement, Artificial Test Soils (ATS) and other methods are being trialed in order to provide a validated basis for cleaning and hygiene performance standards (Griffiths 2000, Boyce 2009). It is important that measure which provide a numerical reading are appropriately qualified and validated so as to provide a meaningful data set which indicate clearly, accurately and with good repeatability the actual hygiene status of the surface to be examined.
We took three commercially available hand held instruments used to measure ATP as an indicator of soiling presence on a surface. We also used two versions of a chemiluminescent Artificial Test Soil (Carling 2006). Each system was subjected to a qualification process for validation as per ISO 13485:2008 and the ICH Guidelines (ICH 2005).
Results demonstrated that whilst the ATP hand held units have reasonable performance across a log scale, variability across a narrow range of data is variable and repeatability may be difficult to achieve.
These findings may undermine some publications where interpretation is based on data without linearity within the range of the tests being undertaken. The results highlight the need to thoroughly understand a device prior to purchase and to ensure that readings are interpreted with some care.

Methods

ATP Hand Held Units Validation

Three commercial brands of ATP hand held measurement devices were selected from brands available in the Australian market. The three brands were Biotrace (3M); Lumiscan (Kikkoman), and Ultrasnap (Hygiena). Each of the brands were used with their own dedicated surface sampling consumables. All three brands of consumables were tested close to the end of the stated shelf life of the consumables to represent a worst case outcome as intended in a sampling system under ISO 13485:2008. ATP was sourced from SIGMA laboratories as an individual reagent and also within a Adenosine 5'-triphosphate (ATP) Bioluminescent Assay Kit. Storage of the ATP was as per recommendations (<20^{o}C) until use. Standard solutions were made up as per instructions and buffered to pH 7.8. All stock solutions/reagents were stored in ice until immediately prior to use. Using a calibrated microtitre pipette precisely 10µL of the ATP solution was placed directly onto each swab. The swabs were aseptically re-capped and reactant solutions released as per instructions and mixed for 15 seconds and then placed into the ATP meter. Readings were recorded. All readings were taken in quadruplicate with some samples repeated on five separate occasions (outliers). Readings of individual swabs were regularly reconfirmed to ensure individual reading repeatability. All ATP readings were calibrated using LC-MS (HPLC mode).

Artificial Test Soil – ATS Chemiluminescent Validation

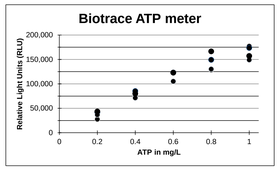
Original ATS Chemiluminescent marker was sourced from Dr Carling and recently released DAZO spots were sourced from Ecolab. Each type was subject to drying and naked eye visibility measurements as well as definitional description under UV light. Results were recorded and photographed.

Results

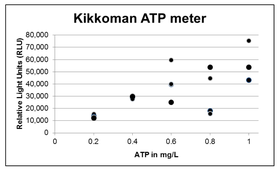
ATP Readings

Higher concentration (< 1mg/L)

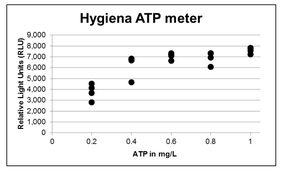
Biotrace



Kikkoman

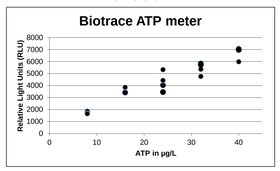


Hygiena

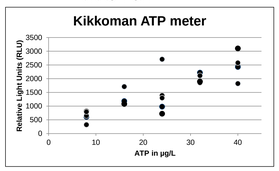


Lower concentration (<0.1 to 40 µg/L)

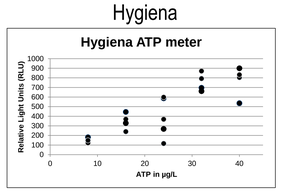
Biotrace



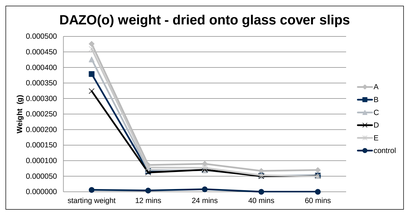
Kikkoman



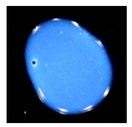
Hygiena



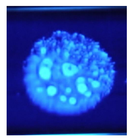
ATS Chemiluminescent Spot Markers

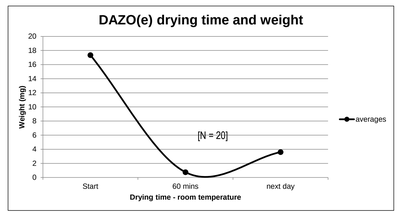


DAZO (o)



DAZO (e)





Discussion

An essential component of any quality driven approach is accurate information. The standard approach to ensuring accurate information within industrial and healthcare settings is validation. Full and proper validation ensures that the information provided will be accurate and defensible on each and every occasion that information is required and measured.
Our experiments sought to provide a basic validation of the two newest forms of cleaning monitoring systems, these being ATP measurement using commercially available hand held meters (and their requisite consumables), and the chemiluminescent spot marker systems (DAZO or equivalent). The use of these measures is subject to separate consideration. This poster outlines the outcomes purely of the scientific measurements to achieve validation of the two approaches.

ATP: as the normal use of the swab consumables involves a variable other than the machine (i.e. swab technique), it was decided to use the ATP directly onto each swab thus eliminating a variable, and thus to more accurately assess the reliability (repeatability) of the ATP measurement via the luciferase reaction (Boyce 2009). This approach demonstrated that each unit had considerable variability despite the accuracy of the application of the ATP standards. Other issues observed in the data were the exhaustion of the luciferase reaction with the Hygiena Unit at high concentrations of ATP (the reaction levels off). Generally the variability in the Kikkoman device was poor within the chosen dilution series.

ATS: both versions of the DAZO chemiluminescent markers dried effectively although the DAZO(o) [original] had greater mass, took longer to dry and also exhibited some evidence of hydroscopicity. The DAZO(o) had a much smaller footprint, with well defined boundaries, whereas the DAZO(e) had a larger footprint and less well defined margins.

Conclusion

ATP: Using a pure solution of ATP allowed the validation process to demonstrate the lack of linearity and repeatability for the three hand held ATP meters. Two brands performed more erratically under these test conditions. The Biotrace Unit uses a Photo-multiplier within the luminometer where as both the Hygiena and Kikkoman units make use of Photo-Diode Array technology (Griffiths 2008).
It is essential that the units and scale of measure have separation between different known levels of ATP present. This allows the more contaminated areas to be clearly identified from the less contaminated areas. This is important as it saves time in unnecessary rectification work by cleaning staff.
All three brands of ATP units demonstrated a lack of separation between known levels of ATP. This undermines the reliability of any individual reading. In our experiments readings were conducted in quadruplicate to allow for calculation of median values. Even so, the variability was particularly high for the Kikkoman unit.
For any use of these units in the field, it is very hard to see much costs savings as readings will best be conducted in quadruplicate in order to minimise variability.
DAZO: each of the two types of chemiluminescent marker performed reliably and repeatedly. The slightly hydroscopic nature of the original DAZO(o) was not a diminishing feature. Both versions yellowed over time but neither were ostensibly visible. Both versions were validated as suitable for use with repeatability in application, appearance and removal. This system has been demonstrated well in the literature (Carling 2008).

Acknowledgements

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The problem of rapid ATP systems may be scaling using Relative Light Units (RLU)

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University of Western Sydney
Bringing knowledge to life

Introduction

The use of rapid Adenosine Triphosphate (ATP) bioluminometers in the monitoring of surface hygiene as originally suggested by Griffiths has yet to see practical fulfilment amongst healthcare infection control and prevention practitioners. (Griffiths 2000) Reviewers have noted the potential for the use of ATP in providing near instantaneous results on quantitative measurements of surface hygiene. (Carling & Huang 2013; Mitchell et al 2013) Despite a large number of studies, authoritative sources have declined to declare ATP monitoring has sufficient evidentiary support for regular use in the monitoring of surface hygiene within healthcare settings. (Loveday et al., 2014)

Rapid ATP bioluminometry has a reliable capacity to distinguish between ten-fold serial dilutions of ATP and various common bacterial species. (Aitken 2011, Whiteley 2012) Brand to brand benefits have been suggested in controlled studies in the endeavour to pick winners, despite clear problems with sensitivity within the scaling. (Andersen 2009, Sciortino 2013; Malik 2013)

There have been problems noted in use of ATP monitoring including unexpected and unusual results that often presented, such as readings of ATP which were higher following cleaning than measurement prior to cleaning being conducted. (Cooper 2007) The correlation between contamination due to microorganisms of concern often correlates poorly with ATP measurement which tend to be higher. (Smith et al., 2013; Boyce 2011) Unexplained variability has impeded efforts to identify a suitable benchmark. (Lewis 2008, Moore 2010, Anderson 2011) Even collection of sufficient data and application of a receiver operating curve model has not resulted in a standardised and widely accepted definitional boundary on hygiene based on ATP measurements expressed as Relative Light Units (RLU). (Mulvey 2011, Smith 2013)

In response to these attempts at solidifying a suitable cleanliness benchmark using rapid ATP bioluminescence, conceptual concerns have been highlighted with ATP as a surrogate for surface hygiene assessment, particularly noting the issues arising from variability. (Malik & Shama “20 pence” JHI 2011) The coefficient of variation has been noted as an problem with ATP monitoring, subsequently poor correlation (r^2 was 0.078). (Shama & Malik IJHEH 2013)

A controlled laboratory model indicated that whilst rapid ATP bioluminometers could distinguish well between ten-fold dilutions, all of the systems tested struggled with a 20% dilution series. (Whiteley 2012) Coefficient of Variance (CoV) used to normalise data sets has shown variance as a substantial issue in using rapid ATP monitoring. (Whiteley 2013)

Uncontrolled variability in data sets is a major problem for statistical applications. (Lautenbach 2012)

In this work, the variability of 4 commercial brands of rapid ATP bioluminometers were tested in a series of laboratory studies using a standardised pharmaceutical industry approach (ICH 2005). The four brands of ATP bioluminometers and their consumables used in this work were 3M – Cleantrace; Kikkoman – Lucipac-pen; Hygiena – Ultrasnap; and Charm – Pocket Swab Plus. A calibrated HPLC using a fully validated method for ATP detection was used as a control for variability.

Method

ATP Testing

In this series of experiments, a known source of ATP (Sigma Aldrich, Castle Hill, Australia) was used to assess ATP device response. The quantitative aspects of the concentration of the ATP were confirmed using HPLC (Shimadzu, Japan) in a validated method. The method of application followed the prior published method. (Whiteley 2012)

Experimental work demonstrated that direct application by micro-titre pipette of 10 – 20 µL of ATP , stored in ice during the experiment, provided a robust, repeatable and defensible method of application that removed other error from the work. At each dilution point a minimum of three swabs were used for each device and wherever possible test points were sampled in quadruplicate.
The use of medians was preferred in range finding as the use of averages was found to be affected by the regularity of outliers in the sampling groups.

The ATP work was repeated on multiple occasions, with multiple operators present. All swabs were used prior the recommended expiry period. All swabs were stored in below 5oC until prior to use when they were allowed to reach room temperature. Multiples of differing batch lots were tested.

The Charm device and consumables were obtained for testing towards the end of the study, and so the number of sample points (runs) and total number of swabs used is somewhat less than the other three brands.

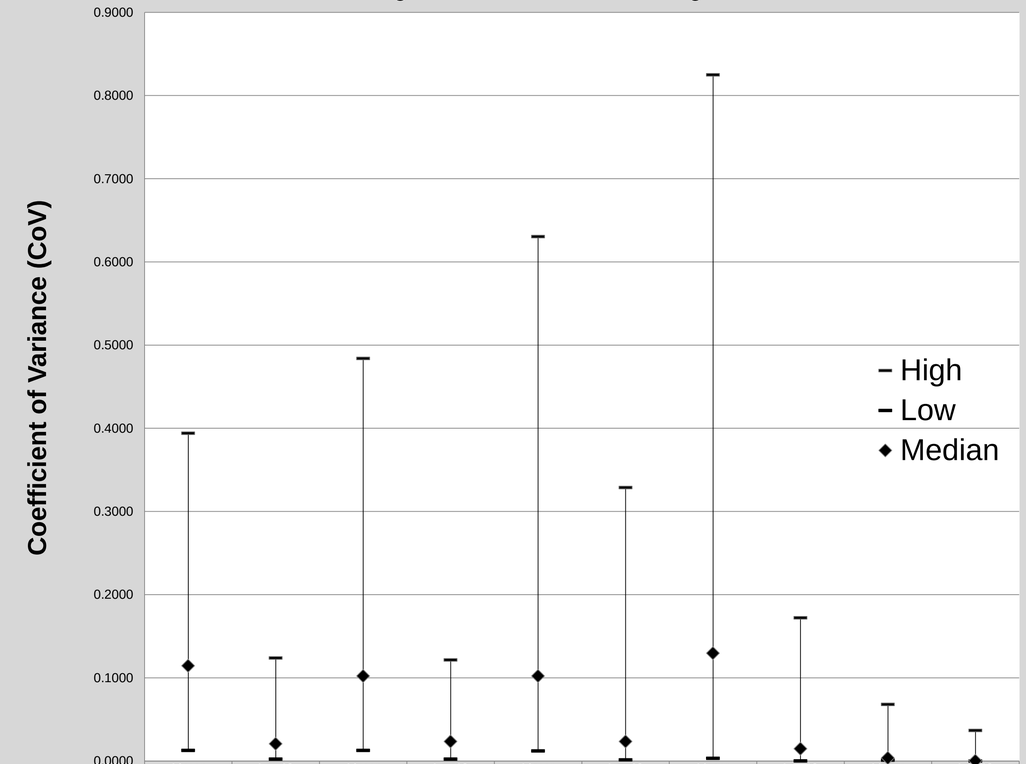
Statistics and Graphs

The ATP readings are made in Relative Light Units (RLU). Each branded device has its own unique numerical presentation and scaling for RLU. No two units share a common scaling.

In these studies the RLU were noted for each data point and the mean, standard deviation and Coefficient of Variance (CoV) were calculated for each sampling run. The raw data was then converted to its log 10 and the statistics were re-calculated using MS Excel (Microsoft Corp, Seattle, USA). The graphs was also drawn using MS Excel.

Figure 1: Relative Light Unit (RLU) scaling shown as both linear and Log10: Co-efficient of Variance (CoV)

The data on variance is plotted as range data with the median noted on each data set



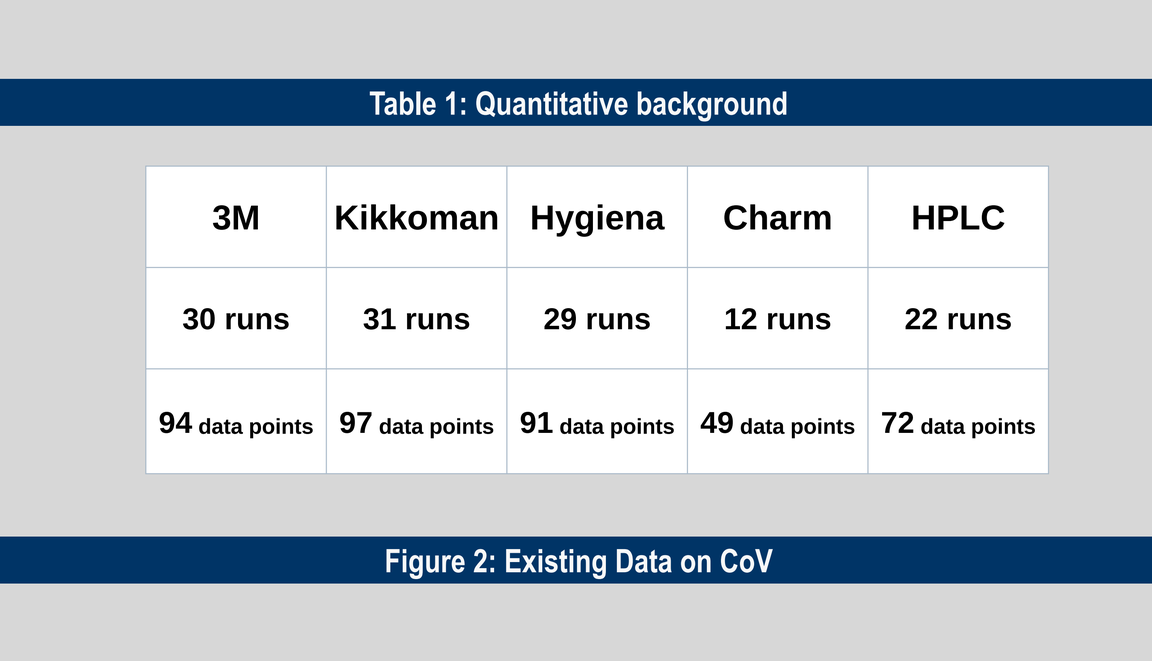
	3M		Kikkoman		Hygiena		Charm		HPLC	
	Linear	Log 10	Linear	Log 10	Linear	Log 10	Linear	Log 10	Linear	Log 10
High	0.3941	0.1239	0.4840	0.1215	0.6304	0.3287	0.8249	0.1721	0.0681	0.0369
Low	0.0128	0.0023	0.0128	0.0024	0.0121	0.0013	0.0033	0.0002	0.0011	0.0001
Median	0.1145	0.0208	0.1022	0.0236	0.1022	0.0236	0.1296	0.0148	0.0037	0.0004

Table 1: Quantitative background

3M	Kikkoman	Hygiena	Charm	HPLC
30 runs	31 runs	29 runs	12 runs	22 runs
94 data points	97 data points	91 data points	49 data points	72 data points

Figure 2: Existing Data on CoV

Showing the Coefficient of Variance (CoV) as a dot plot clearly demonstrate that portable ATP meters do have the same level of reproducible reliability and robustness as a fully validated HPLC. The current methodological approach for ATP measurement, using field based, hand held devices cannot be expected to possess accuracy at the level of a stabilised analytical system. The variance displayed by all brands is much higher than might otherwise be expected as seen in Figure 2 below.



Discussion

The problem of variability with use of rapid ATP bioluminometers has been overlooked in published work and is invisible where single point sampling is undertaken without duplicate, triplicate, or preferably quadruplicate sample measurements. (Whiteley 2014) Thus variability in the use of rapid ATP bioluminometers is the most recurrent technical concern for cleaning monitoring studies within healthcare applications using quasi experimental frameworks.

The risk of type I errors due to unrepresentative sampling in environmental monitoring worsens if the ATP devices used for monitoring have uncontrolled variability. (Martin 1994)

In this work the variability which has plagued so many of the published studies using rapid ATP monitoring has been fully investigated and defined. The four brands used in the study have their own characteristics which form the basis of brand to brand differences, but it an up to individual preference when making a purchasing decision. (Griffiths 2008) What this study has clearly indicated is that variability in the readings obtained require careful consideration when developing sampling plans for use of ATP monitoring within healthcare settings.

The findings on variability in the use of rapid ATP bioluminometers appear to be uniform across all of the brands tested with CoV results showing no difference between brands, either in the spread of variance or clustering of CoV outcomes. The implications of this work are directly relevant to how cleaning monitoring with ATP bioluminometers is conducted and the impact on any statistical measures to be applied to the findings. (Whiteley 2014) As we have noted above, the use of median results provide a more consistent platform on which statistical findings could be based, thus suggesting a discontinuance of averages as an approach to sampling standardisation with ATP. This is particularly important at the low end of the dynamic range where the issue of a practical zero is applied to ensure low end conformity of data.

The alteration of the scaling from its normal linear scale into the log10 of the original data and then recalculating the CoV illustrate the problem with the current scaling approach. As seen in Figure 1, none of the ATP devices compare favourably with the HPLC, although when the data is in the Log phase, variability (CoV) is reduced to a manageable level.

Conversion of the current scaling into a single and unified scale that is in the log phase, rather than the current disassociated and non-unified Linear RLU scale will be highly desirable to reduce variability and improve practicability in rapid ATP testing. Further studies are underway.

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Evidence based cleaning and cleaning validation using FM and ATP systems

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Introduction

Cleaning is a process that when conducted efficiently and effectively can reduce the risk of cross infection via surfaces within healthcare settings (Popp 2013). The four monitoring tools available for monitoring of environmental cleaning are: (i) visual inspection; (ii) microbial recovery; (iii) Fluorescent Marker products (FM); and (iv) rapid ATP (Adenosine Triphosphate) bioluminescence (Carling 2013, Mitchell 2013).

Australia has moved to a quality driven process through the adoption of the National Service and Healthcare Quality Standards (NSHQS) introduced in 2012, which have the two pronged goal of standardisation and continuing improvement (ACSHQ 2012) including the risk of infection associated with environmental cleaning failure. This study investigated the use of Failure Mode Events Analysis (FMEA) as a risk analysis tool in the monitoring of cleaning processes using rapid ATP and FM.

When conducted thoroughly, the cleaning process has been shown to reduce environmental contamination and also to reduce risks of cross infection (Hota 2009, Dancer 2009, Friedman 2013). When cleaning is conducted poorly, inefficiently or ineffectively, the outcome will produce a hazard element and provide a risk platform for cross infection within healthcare settings (Dancer 2012).

The difficulty in developing a clinical approach to cleaning verification is highlighted by the recent comment that that 'cleaning is a "weak science" based usually on a poetic theory not grounded in fact' (Harbath 2013). This highlights the need to take a risk based methodology to link the underlying and sometimes disjointed sciences into a cohesive "process control" approach.
The significant problem with environmental "cleaning" is twofold.

Firstly it is a relatively straightforward process of wiping and removing soils from a surface. Secondly however, cleaning is also a multi-faceted process and not just one science or a single scientific discipline. It is a complex array of linked activities that involve many scientific inputs to arrive at a single desired output (a clean surface). Human factors add a tertiary layer of complexity in the application of the task of cleaning (Jackson 2014).

The total approach to cleaning validation remains poorly studied or little understood (Gillespie 2013). In Australia, various auditing approaches and measurements have been used to indicate cleaning performance incorporating the verification tools available (Victorian Government 2005, NHMRC 2010, Murphy 2011). Moving to a "process control approach" within a standards framework is required to achieve management control overall cleaning process outcome.

The analysis of how bring these diverse issues together and to insert the use of the cleaning verification tools into the cleaning process was considered as per Australian Standard AS/NZS 31000:2009, "Risk Management – Principles and Guidelines" (Standards Australia, 2009). This standard outlines the principles for conducting an overall risk management process. For cleaning, the primary risk is failure of the process. For this study we focused solely on the cleaning verification tools on the basis that if the verification tools fail to work, then underlying cleaning failure will not be identified and corrected, leading to overall process failure.

The use of FM is a validated method of checking that surface cleaning/wiping has occured (Carling 2008). The use of ATP has been shown to correlate well with high levels of bacterial pathogens on the surfaces found within healthcare settings (Smith 2014).

Our team selected FMEA as the most appropriate tool for our study FMEA as a risk assessment tool has been used or suggested on a number of occasions in the field of infection prevention but has not gained any wide spread acceptance (Monti 2009, Chiozza 2009).

The greatest use of FMEA as a semi-quantitative risk assessment tool is in the pharmaceutical and medical device sectors (van Leuwwan 2009).

Method

The two identified cleaning verification methods of rapid ATP and FM were assessed by the Risk Assessment team members to identify the structural or procedural failure modes.

FMEA is a structured methodology which provides a quantitative predictive assessment of a process so as to prevent problems or failures before they occur (McDermott 2009). FMEA looks at the process and predicts possible failure modes. This identification of failure mode is by type so that subtle variations can be assessed together as they will provide a similar failure outcome.

A small multi-disciplinary team was convened to consider and risk assess failure modes of the two cleaning monitoring tools, ATP and FM.

FMEA also allows for the team members to note both the existence of underlying redundancy in the risk assessment (i.e. is the risk already overcome by an existing factor not clearly seen in the risk assessment for the characteristics). The possibility of further intervention can also be noted to indicate any observations as to the best way to lower or mitigate the identified risks.

The method used by the risk assessment team considered the major clinical failures a structured approach to consider worst possible failure event outcomes.

Mitigation was actively considered following identification of key failure modes and interaction with possible redundancy factors.

Results of FMEA (structural) failures

The results of the first stage of failure mode are shown in Figures 1 and 2 below, in regards to drying of the FM. Results of the validation work on ATP systems are shown separately (Poster 3133).

DAZO original and DAZO (e) both take several minutes to dry. In the period of time when drying is underway, the risk of inadvertent removal is quite high as the intention is to apply the FM dots to High Touch Objects or surfaces. Early removal other than by intentional cleaning cannot be detected and allows for a false reading. Our work identified this failure as a False Positive result (the intention is to remove the FM through a cleaning/wiping process). This is a high risk failure as it is not able to be mitigated in the normal course of cleaning activity. FM is noted to only provide qualitative information on cleaning efficiency and not cleaning efficacy. This failure is noted in Figure 3.

For ATP the significant failure risk identified is that the ATP is read and the RLU outcome is below the intended threshold. But if pathogenic organisms remain on the surface, or the ATP reads low due to a lack of detection then the low RLU is a false indication of surface cleanliness. Our work identified this failure as a False Negative result (the intention is to detect unclean surfaces through high RLU readings and a low RLU reading when surfaces remain contaminated with pathogenic and multi-drug-resistant-organisms [MDRO] is a false negative result). This failure is noted in Figure 4 below.

Results

Figure 1: Drying of DAZO original

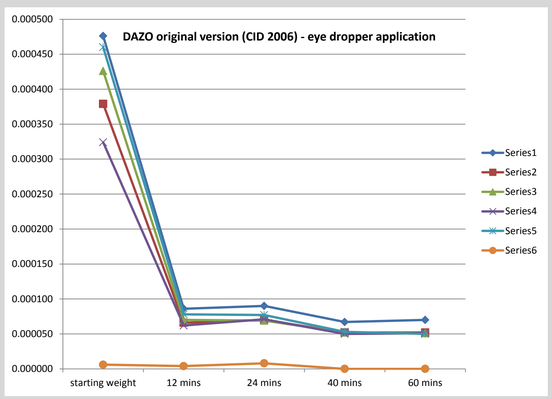


Figure 2: Drying of DAZO (e)

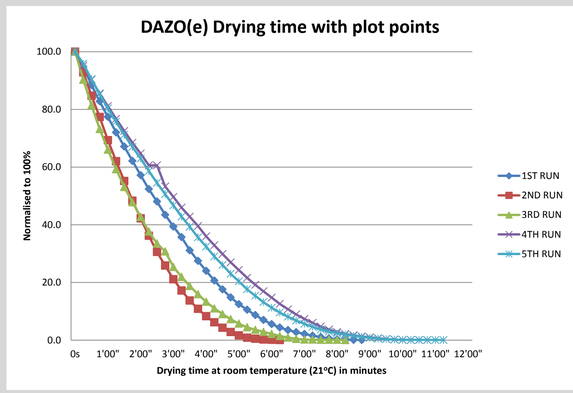


Photo 1: DAZO original

Photo 2: DAZO (e)

Results of FM laboratory studies

Figure 3: Identified failure profile with FM

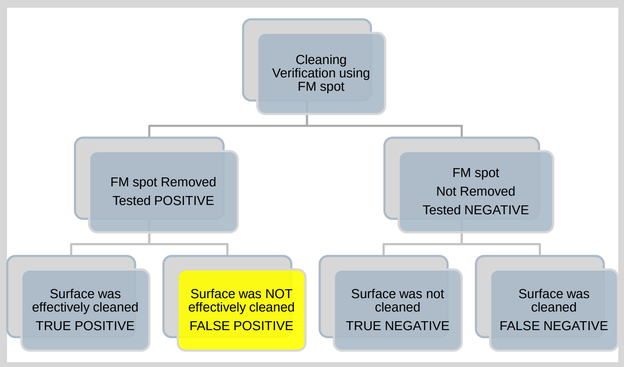
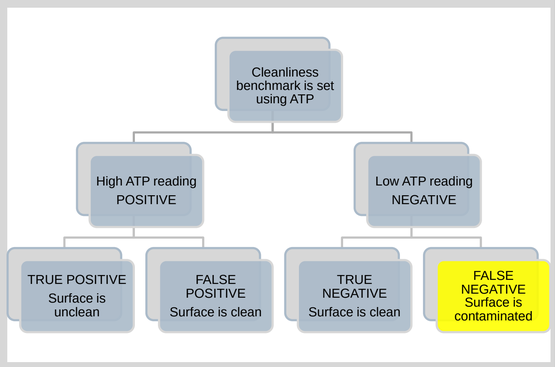


Figure 4: Identified failure profile with ATP



Revised Management Approach based on Failure Mode Effects Analysis (FMEA)

Figure 5: a revised approach to use of FM and ATP in cleaning monitoring

Discussion

The risk assessment of any cleaning process should consider not just the overall risk, but each of the individual sub-tasks and the risks attached to each. For our study we focused solely on the cleaning verification tools and the risks associated with each tool, on the basis that no matter what the cleaning materials and methods chosen, if the verification tools fail to work, then underlying failure will not be identified and corrected, leading to overall process failure.

This FMEA indicates that use of FM has a low risk of failures but also is qualitative and does not indicate the quantitative efficacy achieved through the cleaning process. Whilst ATP bioluminescence measurements can indicate the quantitative level of surface hygiene, the failure modes identified suggest that it should be carefully managed within a risk based system (Malik 2003, Malik 2012).

The results presented indicate that the FM and the ATP should be used both in parallel and in sequence to mitigate the substantial failure risk associated with these cleaning monitoring tools.

This study has focused on the overall failure risk as informed by FMEA. A full study has been submitted for publication and is currently in peer review. The results here are the preliminary and overall impacts of the two most significantly identified failure modes foe both FM and ATP.

The overall impact of using both FM and ATP to mitigate each other provides a new approach for cleaning monitoring within healthcare settings. Further studies are currently underway to further investigate the potential of overcoming the well recognised flaws in hygiene science within hospitals globally.

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A new method for practical quality control in monitoring the cleaning of healthcare surfaces

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Bringing knowledge to life

Introduction

Cleaning within healthcare settings is an intervention that is both practical and cost effective. Quality assurance over cleaning remains problematic with four monitoring methods being visual inspection, microbial recovery, Fluorescent Markers (FM) and ATP bioluminometry (ATP) (Mitchell 2013).

When conducted thoroughly, the cleaning process has been shown to reduce environmental contamination and also to reduce risks of cross infection (Hota 2009, Dancer 2009, Friedman 2013). When cleaning is conducted poorly, inefficiently or ineffectively, the outcome will produce a hazard element and provide a risk platform for cross infection within healthcare settings (Dancer 2012).

In Australia, various auditing approaches and measurements have been used to indicate cleaning performance incorporating the verification tools available (Victorian Government 2005, NHMRC 2010, Murphy 2011). Moving to a "process control approach" within a standards framework is required to achieve management control overall cleaning process outcome.

The use of FM is a validated method of checking that surface cleaning/wiping has occured (Carling 2008). The use of ATP has been shown to correlate well with high levels of bacterial pathogens on the surfaces found within healthcare settings (Smith 2014). The variability identified with ATP use can be overcome through appropriate sampling methodology (Whiteley 2015).

Our team selected FMEA as the most appropriate tool for our study as it allowed a comparison based on the risks of failure, rather than a direct association (Whiteley 2015).

This poster demonstrates the results of investigations into the cleaning monitoring processes. A new method of integrated assessment for cleaning monitoring is proposed.

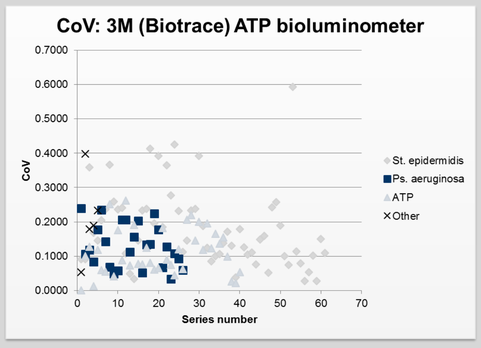
Method & Results

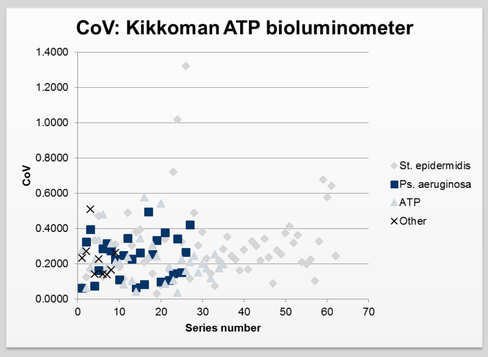
ATP Testing

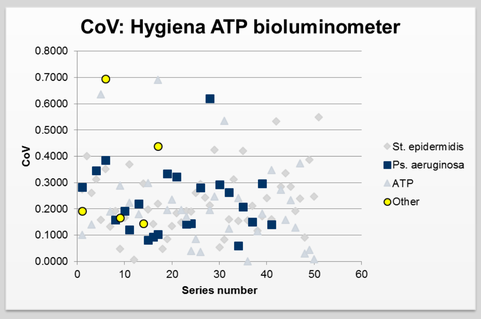
In this series of experiments, a known source of ATP (Sigma Aldrich, Castle Hill, Australia) was used to assess ATP device response. The quantitative aspects of the concentration of the ATP were confirmed using HPLC (Shimadzu, Japan) in a validated method. The method of application followed the prior published method using direct application by micro-titre pipette . (Whiteley 2012) At each dilution point a minimum of three swabs (usually four swabs) were used for each device. The ATP work was repeated on multiple occasions, with multiple operators present. All swabs were used prior the recommended expiry period. All swabs were stored in below 5oC until prior to use when they were allowed to reach room temperature. Multiples of differing batch lots were tested. The Charm device and consumables were obtained for testing towards the end of the study, and so the number of sample points (runs) and total number of swabs used is somewhat less than the other three brands.

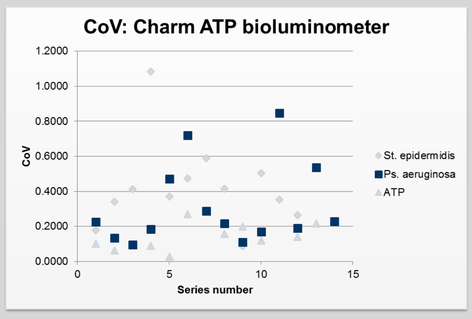
Statistics and Graphs

The ATP readings are made in Relative Light Units (RLU). Each branded device has its own unique numerical presentation and scaling for RLU. No two units share a common scaling. In these studies the RLU were noted for each data point and the mean, standard deviation and Coefficient of Variance (Cv) were re-calculated using MS Excel (Microsoft Corp, Seattle, USA). The graphs were drawn using MS Excel. Cv was preferred for the normalisation of the data given that each concentration was individual and not comparable.

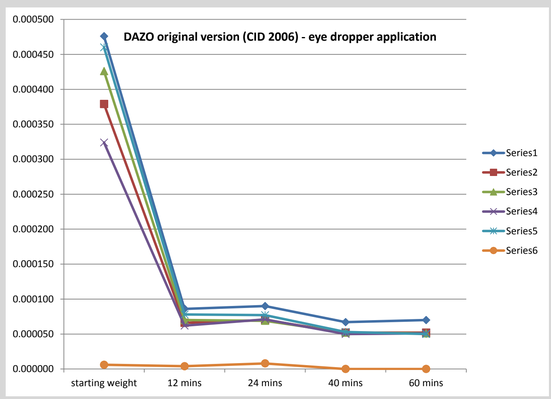








Fluorescent Markers (FM)



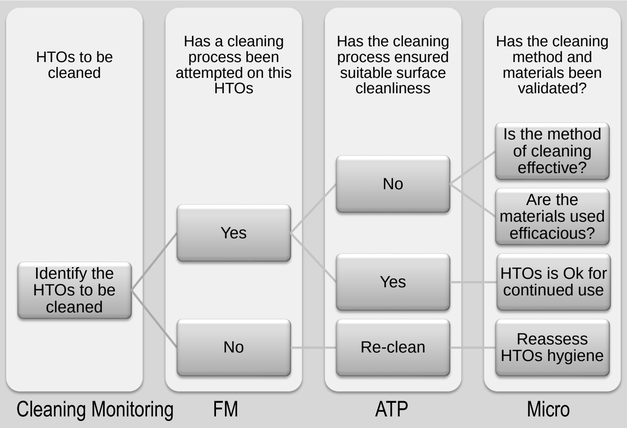
For the purposes of the FMEA study, our work focused on the DAZO system developed by Dr Phillip Carling. The drying time was identified as a important characteristic for field based use so a series of laboratory based drying studies were undertaken to establish the drying characteristics to be used for the FMEA comparison. Drying with the second generation series (DAZO – Ecolab) are shown.

FMEA Key Failures identified

	Failure Mode	Risk Class	Mitigation suggestions	Mitigation Option
Visual Inspection	Surface looks clean but is contaminated with pathogenic microbes or other soils	High	Testing of surfaces with a quantitative method for determining surface hygiene may indicate contamination e.g. ATP testing or Microbial Recovery	ATP or Micro
	Surface looks clean but has not been subject to routine cleaning process[12]	Medium	Use of an FM technology will indicate surface cleaning frequency rate.	FM
Microbial recovery	Result from sampling is delayed by 48 hours or more[29]	High	Testing of surfaces with a rapid method (e.g. ATP testing) can inform and guide the sampling processes by indicating any loci of contamination through use of ATP	ATP
	Pathogenic microbes are not detected on swab but are present on HTOs[10]	Medium	Validation of the sampling methods can improve microbial recovery rates - check cleaning with FM	FM
ATP bioluminometry	ATP Detection failure (Possible lysis system failure), or insufficient bacteria recovered	Medium	Microbial recovery can be used for confirmatory quality control where FM indicates inadequate cleaning but ATP readings are inconclusive	FM or Micro
	Failure to detect viruses due to absence of ATP	Medium	Use of an FM technology is low cost and will indicate surface cleaning frequency in additon to use of rapid ATP detection. A suitable microbial recovery method for virus detection could also be used.	FM or Micro
FM technologies	FM spots are visible to cleaning staff and are preferentially removed	Medium	Testing of surfaces prior to use of FM and coincidental use of ATP will indicate surface hygiene	ATP
	FM assessment does not enable measurement of the level of effectiveness of the cleaning process	Medium	ATP measurement will provide an adjunct measurement of surface cleanliness	ATP or Micro

FMEA is a structured methodology which provides a quantitative predictive assessment of a process so as to prevent problems or failures before they occur (McDermott 2009). FMEA looks at the process and predicts possible failure modes. This identification of failure mode is by type so that subtle variations can be assessed together as they will provide a similar failure outcome.
A small multi-disciplinary team was convened to consider and risk assess failure modes of the two cleaning monitoring tools, ATP and FM.
FMEA also allows for the team members to note both the existence of underlying redundancy in the risk assessment (i.e. is the risk already overcome by an existing factor not clearly seen in the risk assessment for the characteristics). The possibility of further intervention can also be noted to indicate any observations as to the best way to lower or mitigate the identified risks.
This table presents a snapshot of the two worst failure modes for each of the monitoring methods (Whiteley 2015).

Answering the key validation questions



The FM drying is achieved within a practical times of less than 2 minutes, although full drying can take up to 12 minutes depending on the brand. Newer brands dry virtually instantly. The ATP tests lack precision but are robust when considered over a wide range of contamination. Each of the four cleaning monitoring methods has risks which, in isolation, limit their usefulness. FMEA highlights overlapping of weaknesses with strengths, suggesting a new integrated approach to cleaning monitoring within healthcare.

Conclusions

Cleaning is a feature of hospital infection control but lacks a robust mechanism to achieve the desired quality assurance over cleaning outcomes. The Australian Healthcare System endorses a risk based approach to the prevention of healthcare associated infections, including surface hygiene within hospitals (ACSQH 2011). Our study focused on the cleaning verification tools and the risks associated with each tool, on the basis if the verification tools fail to work, then underlying failure will not be identified and corrected, leading to overall process failure.

Further studies are currently underway to further investigate the potential of concomitant use of the four cleaning monitoring methods. The results presented indicate that the four cleaning monitoring methods should be used synergistically to mitigate the substantial failure risk associated with any one of the individual cleaning monitoring tools when those tools are used in isolation.

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DIsclosures

Disclosure statement:

None of the authors have any connection to any of the materials studied or presented in this study. This work has been completed as part of studies toward a PhD by the principle author. There is no affiliation with any 3rd party mentioned as part of this study. GS Whiteley and Dr T Glasbey are both employees of Whiteley Corporation. Dr Derry is a Senior Lecturer at UWS.

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