American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Research Article

Is There a Measurable Relationship Between Air and Surface Contamination? Study Of Student Classrooms and Sports Facilities at the University of Surrey, UK

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To Cite This article: *Andrew Kemp JP, PhD*, D Hanson FBICSc, Is There a Measurable Relationship Between Air and Surface Contamination? Study Of Student Classrooms and Sports Facilities at the University of Surrey, UK. Am J Biomed Sci & Res. 2026 29(5) AJBSR.MS.ID.003850,*

DOI: 10.34297/AJBSR.2026.29.003850

Received: January 06, 2026; **Published:** January 21, 2026

Abstract

The British Institute of Cleaning Science, in association with the CSSA and Surrey University undertook a study to determine if there is a quantifiable relationship between air and surface contamination levels in the University classrooms, bathrooms and sports facilities.

In total, 740 bacterial cultures using blood agar plates, 740 live bacteria specific rapid metabolic assays (BSRMA) [1] measuring Colony Forming Units (CFU's), and 8,400 air particle count samples (APC's) of between 0.5 and 1 microns, were taken from 10 busy rooms (including classrooms) in the University of Surrey. One room was used as a control with no changes made from their normal routine cleaning regimes, disinfectants and cleaning materials. The rooms treated were blinded to the cleaning operatives. Prior to any changes, rooms were tested to give base readings after routine standard cleaning, and before the start of the working day. Rooms were tested again after 4-6 weeks and after 10 weeks, over an academic year. This 10 room study included a sub sectional study using 3 rooms and the addition of new disinfecting technologies for both the air and surfaces, to see if the test results altered when these technologies were used.

In addition, due to the work underway by the UN and WHO to set air indoor quality standards using CO_2 levels as a determining risk factor [2], the relationship between air and surface decontamination techniques and CO_2 levels, were tested by Professor Prashant Kumar's team from the Global Centre for Indoor Air Quality Testing, also based at the University.

The results showed that a direct correlation of approximately 10:1 surface CFU counts to air particle counts of between 0.5 and 1 micron, could be drawn between the air counts taken 20 cm's above the surface, and surface counts of live bacteria. The study also showed that significant improvements in both air and surface counts could be gained, due to the introduction of new cleaning technologies, however these did not affect the CO_2 levels which remained stable throughout.

Background

The original study was intended to test 2 aspects of cleaning. The use of robotics to reduce bioburden and improve cleaning in some key areas, and the use of new disinfecting technologies to reduce airborne and surface bio burden. Unfortunately, due to operational issues within the University FM department, in the final analysis the robotic area results could only be used to supplement the new technologies data in looking at the relationship between airborne and surface counts.

There are numerous papers showing the significance of surfaces in the potential for cross infection [3,4]. With Antimicrobial resistance (AMR) on the rise, and a proven link between antibiotic resistance, and disinfectant resistance [5], it is now more than ever, imperative that we test surfaces in hospitals regularly, to determine levels of contamination. To date, there is still no internationally agreed definition of what constitutes resistance to disinfectants and sanitisers. In addition, there are no international standards or even country standards, recommending which surfaces in hospitals should be sampled for bioburden, how often, and what test methodologies should be used [6]. In fact, as we don't routinely test surfaces, there has never been an agreement to produce acceptable standards for what would be deemed to be "safe" levels of contamination on surfaces in hospitals. Whilst there have been

attempts to engage the UK government to approve standards for environmental cleaning in healthcare, poor leadership from "NHS Improvement" in their most recent standard setting document, left the UK with a significantly watered down practice requirement, and with no requirement for reliable microbial surface or air particle testing [7,8]. The NHS Improvement document does recommend cleaning audits, however, this is provided for by visual inspection only. The recommended practice in the document is at best of no clinical value, and is not based on any data or evidence.

For each of the currently available test methods, whilst there are published standards for air filtration in the UK [9], there are no standards to determine acceptable levels of bioburden for surfaces in diverse areas such as the Operating Theatre, general ward, or the hospital admin offices. Clearly, the common sense approach, is to assume that lower the bioburden in both air and on surfaces, the better it is for patients and staff. The questions that require answers are then;

1. Which surfaces should be tested?
2. Which test methods should be used?
3. How often should surfaces be tested?
4. What results are acceptable?
5. When should the test results be a cause for concern/ intervention?

As previously stated, with Anti-Microbial Resistance (AMR) to both disinfectants and antibiotics being on the rise [5] and inextricably linked, a fast, accurate, simple and inexpensive surface test, that measures both CFU's per cm^2 and that can identify species, needs to be made available to healthcare staff.

Of the currently available tests for surface contamination, only one has been peer reviewed, and accepted as specifically designed to test surfaces [10]. The rest were designed for internal medicine and adapted for use on surfaces. As with any products adapted for use in other ways than originally intended, there will understandably be compromises causing limitations on their accuracy, with time delays in results, and therefore usefulness for assessing efficacy of surface cleaning.

If we look again at the ideal criteria for a useful surface test for hospitals, none of the available tests fulfil all the requirements set out below.;

1. Fast - must be in real time, so that dangerous CFU levels and species can be identified quickly, and dealt with before they become a problem.
2. Accurate – must have a level of confidence that the results are correct within an acceptable margin of error.
3. Simple – ideally staff can test their own areas of work responsibility. Whilst specialist equipment is required, it should be simple to learn to use.
4. Inexpensive – If tests are expensive, they will not be used regularly. From a global perspective, in countries where they are cost prohibitive, it unlikely they will be done at all.

"If you can measure it, you can improve it"

One of the main reasons why there are no current standards for surface contamination levels is that, there is still no test available that satisfies all the criteria above. This study is intended to find a way to satisfy as many of the ideal requirements as we can, until a test can be developed that satisfies them all. A test that satisfies all requirements would allow infection control teams to adopt a proactive approach to testing, as opposed to the current reactive approach, "there is a problem, we need to identify what it is and where it is coming from?"

Introduction to the New Disinfecting Technologies

Technology 1- Advanced Photocatalytic Oxidation (APO)

This product is primarily used for active reduction in live microbial activity in the air. It filters the air, whilst also producing and circulating an hydroxyl radical (free radical) anti-microbial aerosol. Before the introduction of this patented technology, for free radicals to effectively kill microbes in the air and on surfaces, the concentration used would have to be above the safe Maximum Exposure Levels (MEL's). This would mean that either significant Personal Protective Equipment (PPE) needed to be worn in the rooms during treatment, or the room would need to be vacated. The manufacturers of the new APO products used in the study, have discovered a method of reducing the concentration to well below safe MEL's, whilst maintaining therapeutic value. This is achieved by reducing the concentration of free radicals, then passing it over titanium dioxide in the presence of UVc light [11-13].

There was at the time of testing some unpublished evidence, that this technology has the secondary effect of reducing live microbial levels on surfaces. This data is now available [14].

Technology 2 – Photocatalytic Solution (PS)

This surface treatment uses similar Photocatalytic technology to the APO product, in that it uses a form of free radical as its active antimicrobial. As a persistent surface treatment, it is applied every 3 to 6 months to clean surfaces, and remains in place until worn away through frictional forces, i.e use. Like any persistent antimicrobial technology, the reapplication schedule is based on the perceived levels of use of the surfaces and may change from surface to surface. At the time of testing, a test is being developed that will show the presence of sufficient antimicrobial to remain therapeutic.

With both technologies, manufacturers recommend that routine standard cleaning should be continued.

Study Design/ Methodology

In the full study, 10 rooms across a range of classrooms and social interaction areas (including 3 classrooms to be used in a sub

sectional study) were selected to be studied over a 12-month period. 2 surfaces were selected in every room, with swabs taken from 20cm^2 areas for both BSRMA and culture (740 in total for each test). 20 x air sampling measurements were also taken, at 20cm above each area (8,400 samples in total). A control room was selected, and all rooms were tested pre any interventions or treatments. The control room continued with the University standard cleaning regime and products. Tests were then conducted at approximately the same time of day, on the same day of the week at 4 – 6 weeks, and at 10 weeks post intervention. The study was blinded to all staff except the cleaning supervisors, who were instructed not to intervene in any cleaning within the rooms to be tested.

The multi particle sampler unit used for air sampling, can determine 6 different particle sizes in any one sample. One litre of air is sucked into the unit over 1 min. Particles are measured in their respective size groups, and a digital read out is taken of each particle size.

In all rooms, surface and air samples were taken at the same sites at every visit. Samples were taken between 6.30am and 7.30 am in all rooms. These times are approximately one hour after the standard cleaning had been completed, and before the rooms began their normal daily routine work. Normal study classes and meetings took place throughout the testing period with up to 15 people use the classrooms at any one time.

The sub section of this study is the part of the study that is reported in Tables 1 to 3 below. The sub section study, was also designed to use the same test methods over a 10-week period, to test the efficacy of the two new technologies described above. The total number of samples in the sub section study was 48 x BSRMA counts and 48 x bacterial species identification cultures, (no viruses or fungi were able to be cultured). Whilst it is agreed APC's are not an exact measure, highly accurate particle size counts are an acceptable method of determining air particle counts by size of particle for approximation of bacterial, viral and fungal counts (See Annex A). It is therefore also possible to determine that increases or reductions in particles of certain sizes would lead to the conclusion that these equate to increases or reductions, at least in part, in bacteria, viral units and fungi in the air [7,10,11].

Rooms of equivalent size with similar footfall, sharing the same ventilation system, were selected. One classroom was used as the control, a second room was treated with both the two new disinfecting technologies already described. Room three had APO only and room four had PS only. The rooms were in use for all except 2 weeks of the 10-week period. The normal surface cleaning regime was continued in both rooms by the same cleaning operatives, using identical disinfecting/ decontaminating chemicals and equipment.

In the rooms with the PS disinfecting technology, the surfaces were treated after the first set of samples were taken only. Treatment was done by spraying the solution onto the surfaces, they were then allowed to dry fully before students were allowed to enter the rooms. The APO units were placed at the back of the rooms away from the entry doors, after the first set of baseline samples were taken. They were activated at level three which is the manufacturers recommendation for rooms of the size to be treated. A notice saying, "do not turn off" with no explanation of what the units were doing, was taped to each of the units.

Surfaces sampled, were comprised of similar materials, allowing for maximum potential to gain comparator results. Whilst standard testing requires samples to be taken from 10cm^2 areas, evidence has shown that on surfaces where BSRMA live CFU counts are low, culture rarely shows any result [3,6,10]. There is therefore a much better chance of getting a result from the larger sample area 20cm2 [1,7,10] which is in fact four times the size of a standard sample area.

Surface samples were taken from 2 areas in each room using sterile Dacron swabs dampened with "Aespetol". BSRMA counts were used to determine "true" levels of live bacterial contamination to within 10 CFU's. Blood agar plate cultures were used for bacterial species identification [12] using the same samples. Twenty air samples were taken from 20cm above both 20cm^2 areas, on flat tabletops using a multiple particle sampler unit. An average was calculated between the 240 samples of air and the 48 BSRMA swabs, to give an overall average of air particles and surface CFU counts within the room [6] at each data point. All 48 culture samples were processed at room temperature (21-23^{0}C) [11,12].

Results/ Data Sub Sectional Study

The tables below show the averaged results of air sampling by particulate size, the averaged BSRMA results CFU per cm^2, and the result of cultures. From air particle and BSRMA testing, there were no individual sample results of note, all were within statistical relevance of the partner tests.

The tables above clearly show a significant reduction in CFU counts per 20 cm^2, and air particle counts by size in the treated rooms. No cultures grew post treatment with either APO or PS in the treated rooms. The cultures that produced results from the pre treatment samples grew predominantly SA + Ecoli, therefore, the air particle counts of most interest are the 0.5-0.7 microns and 0.7-0.1 microns combined (See Annex A). This allows us to directly compare the relationship of the total of these two size ranges, with the CFU results from the surface BSRMA samples.

Further analysis shows that in the control room, the bacterial counts in the air have an average ratio of 9.36 air particles to 100 CFU's when compared to surface CFU counts. In the APO room, the relationship was 11.68 AP's to 100 CFU's, in the PS room it was 12.32 AP's to 100 CFU's, and in the APO and PS combined room it was 10.79 AP's to 100 CFU's. Overall, the relationship is averaged to 11.6 AP's to 100 CFU's.

It is worthy of note, that no cultures grew on samples where BSRMA results were below 75 CFU's per cm^2, or with a combined 0.5-1 micron APC of 2,521particles (Annex A) (Table 1-3).

Annex A: Approximate particle sizes of pathogens of interest to the study.

Species	Microm^3		
SA	0.52	Staph Aureus	
Psu	0.55 to 0.7	Pseudomonas	
Sp	0.5 to 1.25	Streptococcus pneumoniae	
Kl	0.5 to 0.8	Klebsiella	
Hi	0.3 to 1	Haemophilus Influenzae	
Sh	0.4 to 0.6	Shigella	
EC	0.6 to 0.7	E-Coli	
Cp	3 to 4	Clostridium perfringens	
Ca	1.7	Campylobacter	
BC	3 to 4	Bacillus Cereus	
NCG		No culture growth	
	Below 0.5	? Virus	
	Above 10	Fungi	
CO_2	1 Kg = 0.5458m^3		
			1 micron is 10 to the 18th of a cubic meter

Table 1: Shows the results from samples in all rooms prior to any intervention.

Pre 1st				
Intervention				
05/04/2023				
Vets building				
APC's	Control	APO	PS	APO + PS
Rm No	03VSM	01VSM	07VSM	08VSM
0.1 to 0.5 Micm^3	5,788	4,286	5,218	4,242
0.5 to 0.7	2,928	1,865	2,248	2,259
0.7 to 1	1,847	992	1,637	1,326
1 to 2	612	631	603	557
2 to 5	5	77	6	19
5 to 10	3	18	3	4
BSRMA	48,549	31,662	42,569	37,898
Culture	SA + Ecoli	SA + Ecoli	SA+	
Ecoli	SA + Ecoli			

Table 2: Shows the results after 4 weeks.

4 weeks				
Post				
03/05/2024				
Vets building				
APC's	Control	APO	PS	APO + PS
Rm No	03VSM	01VSM	07VSM	08VSM
0.1 to 0.5 Micm^3	3,030	1,124	932	671
0.5 to 0.7	2,496	463	137	128
0.7 to 1	1,310	290	189	118
1 to 2	405	166	106	80
2 to 5	5	36	6	9
5 to 10	2	18	4	8
BSRMA	38,352	6,629	2,975	2,526
Culture	SA + Ecoli	NCG	NCG	NCG

Table 3: Shows the results after 10 weeks.

10 weeks				
Post				
14/06/2024				
Vets building				
APC's	Control	APO	PS	APO + PS
Rm No	03VSM	01VSM	07VSM	08VSM
0.1 to 0.5 Micm^3	3,083	1,353	1,849	794
0.5 to 0.7	1,517	298	182	139
0.7 to 1	1,004	108	147	124
1 to 2	353	44	198	173
2 to 5	6	56	11	27
5 to 10	3	16	4	9
BSRMA	37,189	4,065	2,403	2,222
Culture	SA + Ecoli	NCG	NCG	NCG

Additional Analysis of all Tests

When all the 8,400 AP sample results from the wider full study were analysed against the 740 BSRMA results, the average combined APC to CFU relationship was found to be 9.57 AP's to 100 CFU's, with a range of 8.11 AP's to 13.69 AP's to 100 CFU's.

There was no statistical difference in air counts of VOC's or CO_2 between any of the rooms, including the control room. There were no changes after any antimicrobial treatments of the air or surfaces, showing there is no relationship between CO_2 levels and either actual air or actual surface contamination after treatment with these highly effective antimicrobials.

Conclusion

Although this remains in inexact science, the relationship of approximately 10: 1 significant air particles to CFU levels is easy to understand and utilise as a measurement for Infection control staff. A simple 1 min air sampling test taken 20cm above a surface, showing a result in the 0.5 to 1 micron range, will give a reasonable estimate that there are 10 X that amount of CFU's on the surface directly below the air sampler. If the result shows more than 2,500 APC's in total in that range, there is a high expectation that with a good technique, a culture sample will give a species identification result.

As a result of this study, it is now the opinion of the authors, that there is a clear relationship between air and surface contamination in both directions. Most importantly, as we can now determine that surface CFU counts will be approximately ten times greater than air counts between 0.5 and 1 microns, it is now possible to use a simple air particle sampler 20 cm above a surface to get a reading accurate enough to approximate surface contamination in just a few minutes. This now gives us a test methodology that fulfils all 4 of the original requirements for us to begin to gather enough data to make recommendations as to the safe levels of bacterial surface contamination.

Whilst it clear from Annex A, that there are pathogens of a different particle size to those used for an APC to CFU ratio, only 9 out 840 culture plates produced cultures with species other than E Coli and SA. In practice, these culture results should not be ignored as they may indicate the need for specialist disinfectants, or change of standard disinfectants due to the potential emergence of a resistant species.

In the sub sectional study looking at the two new disinfecting technologies, as the only measurable differences between the study rooms were the interventions undertaken with both the APO and PS technologies, it is not unreasonable to conclude that these interventions were responsible for the changes.

Although the number of tests undertaken in 4 rooms over a period of 10 weeks could be argued to be a small scale study, the results are so compelling, there can be no doubt that the combination of the two new technologies, significantly reduces the live CFU counts on surfaces and in the air to a degree that would almost certainly be considered to reduce risk of cross infection in an indoor environment.

Again, there was a measurable difference in surface counts using the BSRMA tests, as there were no cultures grown in either of the three treated rooms after treatment, it is impossible to know how much difference in potential for cross contamination there is from either surfaces or air. It is of course possible that due to the "Holism" or "Entourage theory" [13] that the individual product efficacy is increased by the combined use with the other product. As such, it is the authors opinion that the most effective way to use the technologies is by combining them. Results also show, air sampling requires a total of more than 2,500 particles in the 0.5 – 1 micron range to produce a culture plate result from a surface swab. Air sampling could therefore also be used to determine which areas of a surface should be swabbed for culture, as the most heavily contaminated areas above 2,500 AP's are probably the areas that will get a culture result. It is also possible that air sampling could now be used for early identification of antimicrobial resistance. If

contamination levels increase significantly on surfaces that have been cleaned, then it is possible that the bacteria on that surface have become resistant to the disinfectant used.

Whilst we can only estimate the viral load in air samples (by particulate size), it is almost certain that during the year there will have been changes in dominant airborne viruses. From student sickness reporting, these included Flu, Coronaviruses and Norovirus. It appears that the viral load had little or no effect on bacterial counts. Although this is not proven, it can be stated that they had no effect on the ratio of bacterial CFU to AP's in the 0.5 – 1 micron range.

The UN/ WHO indoor air quality standards committee need to take these results into account before producing their indoor air quality standards. Any standard that does not allow for the use of antimicrobials, using only CO_2 levels for guidance, will almost certainly not reflect the true levels of contamination and therefore potential for harm in any room.

Authors Comment

It is now the authors view that whilst air sampling will not give an indication of bacterial species colonising surfaces, as such it does not satisfy all the ideal test criteria, it could be adopted as a fast, accurate, simple, inexpensive and effective way to determine approximate total contamination levels. Whilst accurate and approximate are used in the same sentence and would appear to contradict each other, in practical terms, the test is accurate enough to give confidence that the approximate contamination level on a surface needs attention, or not.

There is clearly still a decision to be made over acceptable levels of AP's and surface counts. We know from studies that some pathogens like Ecoli and Norovirus, require only very low levels of contamination [4] to become a potential problem for staff and patients. Further study is needed to understand at what levels surfaces become a danger in respect to cross infection potential, but the methodology will almost certainly need to include more specificity on species identification. Until a new more accurate test method that meets all the criteria mentioned in this paper is developed, there will still be the potential for cross contamination from surfaces.

As the results show an air sampling total count of approximately 2,500 particles/ 75 CFU per cm^2, is the break point at which a culture swab is likely to produce a result, and as it is clear from the study that there are disinfection products that will result in counts lower than that, until more is known, it is the authors opinion that would be the ideal point at which swabs should be taken, and recleaning/ disinfection of the surfaces should take place.

This research was funded entirely by the BICSc and the CSSA, with products and services provided for free by the relevant parties. At the time of publication there are no conflicts of interest.

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