Continuous Monitoring of Aerial Bioburden within Intensive Care Isolation Rooms and Identification of 'High Risk' Activities

L.R. Dougall[a*], M.G. Booth[b], E. Khoo[c], H. Hood[c], S.J. MacGregor[a], J.G. Anderson[a], I.V. Timoshkin[a] and M. Maclean[a,d]

[a] The Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST), Department of Electronic and Electrical Engineering, University of Strathclyde, Glasgow, UK,

[b] Glasgow Royal Infirmary, NHS Greater Glasgow & Clyde, Glasgow, UK,

[c] School of Medicine, Dentistry and Nursing, University of Glasgow, UK,

[d] Department of Biomedical Engineering, University of Strathclyde, Glasgow, UK

Corresponding author:

Laura R. Dougall

ROLEST, Department of Electronic and Electrical Engineering, University of Strathclyde, Royal College Building, 204 George Street, Glasgow, UK, G1 1XW

Email: laura.dougall@strath.ac.uk Telephone: +44 141 548 2376

Running Title:

Variability of Airborne Bacteria in ICU

Summary

Background: The spread of pathogens via the airborne route is often underestimated and little is known about the extent to which airborne microbial contamination levels vary throughout the day and night in hospital facilities.

Aims: This study evaluates airborne contamination levels within ICU isolation rooms over 10-24 hr periods, with the aim of improving the understanding of the variability of environmental aerial bioburden, and the extent to which ward activities may contribute to this.

Methods*:* Environmental air monitoring was conducted within occupied and vacant inpatient isolation rooms. A sieve impactor sampler was used to collect 500 L air samples every 15 minutes over 10-hour (08:00-18:00 h) and 24-hour (08:00-08:00 h) periods. Samples were collected, room activity logged, and the bacterial contamination levels were recorded as cfu/m^3 of air.

Findings*:* A high degree of variability in levels of airborne contamination was observed across all scenarios in the studied isolation rooms. Air bioburden increased as room occupancy increased, with air contamination levels highest in rooms occupied for the longest time during the study (10 days) with a mean value of 104.4 cfu/m^3 and a range of 12–510 cfu/m^3. Counts were lowest in unoccupied rooms, with an average value of 20 cfu/m^3 and during the night.

Conclusion: Peaks in airborne contamination showed a direct relation to an increase in activity levels. This study provides first clear evidence of the extent of variability in microbial airborne levels over 24-hour periods in ICU isolation rooms and directly correlates microbial load to ward activity.

Keywords:

Airborne; contamination; bacteria; air sampling; bioburden; environment

Introduction

It is estimated that 10-33% of hospital-acquired infections (HAI) are transmitted via the air [1], however the role of air as a vector in the spread of infection is less understood. Over a century on, controversy surrounding particle size, transmission characteristics and associated infection risk have led to a lack of airborne infection control strategies in healthcare premises [2].

Airborne transmission is a route for many serious infectious organisms such as norovirus, influenza, SARS, methicillin-resistant *Staphylococcus aureus* (MRSA) and the highly contagious *Mycobacterium tuberculosis;* whilst multi-drug resistant *Acinetobacter* and *Clostridium difficile* have also been identified in hospital air [3]. Air quality standards exist for operating theatres (<180 cfu/m^3 during an operation, and <10 cfu/m^3 during theatre commissioning and in ultraclean theatres) [4], however there are currently no accepted standards for other hospital areas, including ICU which houses arguably the most vulnerable patients.

Microorganisms originating from the human respiratory tract can become airborne by coughing, sneezing or exhaling, and remain suspended in the air for prolonged periods of time, sometimes indefinitely [5-7]. These infectious respiratory droplets can evaporate to droplet nuclei which have the ability to travel long distances on air currents, and be easily dispersed throughout hospital buildings. As such, numerous studies have reiterated that environmental contamination should not be underestimated, with regards to infection transmission directly from airborne dust, respiratory droplets or droplet nuclei, or indirectly once settled onto surfaces [8, 9-11].

The aim of the present study was to assess, for the first time, continuous (10-24 hour) monitoring of the levels of airborne microorganisms in an ICU and correlate changes in airborne contamination levels to room activity, to generate an improved understanding of the airborne microbial load in a hospital setting.

Methods

Setting

This study was conducted in isolation rooms of an ICU between May and December 2017. The Unit has 3 inpatient isolation rooms and a 7-bed open bay. Isolation rooms chosen for sampling tended to house serious burn trauma cases, critical postoperative care patients or potentially infectious patients. Air entering the unit passes through High Efficiency Particulate Air (HEPA) filters. Both occupied and unoccupied isolation rooms, with an area of approximately 25-30 m^2 (5×6 m), were sampled as part of the study. Rooms were maintained at positive pressure, with a temperature of around 20°C, and had no windows that could be opened. Rooms were cleaned daily: domestic staff cleaned the floor, sink, surfaces, bins and ledges, and nursing staff damp-dusted all frequently touched surfaces and equipment. Cleaning was monitored fortnightly by Facilities staff, adhering to NHS Scotland National Cleaning Services Specifications. GRI Infection Control Policies were adhered to throughout [12].

Sample Collection Methods

Monitoring of airborne contamination was conducted using a Surface Air System (SAS) Super-180 sieve impactor active air sampler (Cherwell Laboratories, UK). The air sampler was situated in the corner of the isolation room, approximately 1-1.5m above the ground and sampled the air by actively drawing a pre-set air volume through the sampler. 500-L air samples were collected every 15 minutes over 10-hour (08:00–18:00h) and 24-hour (08:00–08:00h) periods onto non-selective tryptone soya agar (TSA) plates (Oxoid Ltd, UK), favourable for environmental sampling. An activity log was compiled to record room activity that may correlate with peaks in air contamination. After sampling, TSA plates were incubated at 37°C for 48-hours and enumerated. The total number of microbial colony-forming units (cfu) on each plate was corrected for the statistical probability of multiple particles passing through the same hole, by referring to correction tables supplied with the equipment

[13]. The probable count (Pr) was then used to calculate the cfu per cubic metre of air sampled using the equation:

$$X = \frac{\Pr \times 1000}{V}$$

where V = volume of air sampled; Pr = probable count; X = cfu per 1 m^3 of air.

Statistical analysis

Data was analysed using statistical control charts (Minitab v17) to determine data points classed as 'out of control' from the overall dataset of each case study based on rationale by previous work [14]. 'Out of control' observations (flagged in red) are data points >3 standard deviations above the mean and are significantly greater than the mean of the dataset. Analysis of data between case studies was also conducted using one-way ANOVA at the 95% confidence level (Minitab v17).

Results

Airborne bioburden monitoring over 10-h in patient-occupied isolation rooms

Ten-hour monitoring of patient-occupied isolation rooms took place on three separate sampling days from 08:00–18:00h. The first case study (Fig. 1a) involved a 71-year-old male patient, with a post partial pancreatectomy for cancer and multi-organ failure, occupying the room for 8 days prior to commencement of air monitoring. Results (Table I) demonstrate a high degree of variability over the 10-h period, with a mean airborne bacterial load of 64.3±31.8 cfu/m^3, and a minimum of 12 cfu/m^3, and a maximum of 166 cfu/m^3. This maximum (Observation 16 at 11:45h) was statistically classified as 'out of control' and coincided with collection after fresh bed sheets were shaken in preparation of a bed change.

The results of a second case study (Fig. 1b) were generated in a room which housed a 37-year-old male patient with severe community-acquired pneumonia who had occupied the room for 7 days prior. A mean value of 44.1±36.1 cfu/m^3 was recorded. The patient was mobile, talking and subsequently transferred from ICU after completion of the study. Airborne contamination levels remained low and consistent for most of the study (between 10–50 cfu/m^3) from (08:00–14:00h) during which room activity was minimal. The number of people entering the room was low (0-2) as the patient did not require 1:1 care for most of the period. Bioburden levels increased from 10–110 cfu/m^3 in response to the presence of a visitor at 14:00 (observation 25), and remained elevated until their departure (observation 29 at 15:00h). Significantly higher ('out of control') levels were observed when the patient was assisted out of bed, followed by the removal of the bed from the room. This group of activities occurred between 15:45–16:30h (observation 32-35) and resulted in an increase to 166 cfu/m^3.

A third study (Fig. 1c) was conducted in a room occupied by a 75-year-old female patient, admitted to ICU with pneumonia and multi-organ failure and occupied the room for 3 days. A mean of 48.8±20.5 cfu/m^3 was recorded with a range of 20–116 cfu/m^3. 'Out of control' levels occurred due to a high level of room activity during patient re-intubation, involving an increase from 2 to 4 staff within the room and a higher degree of physical movement around the patient's bed (Observation 9; 10:00h).

Overall, airborne bioburden data (Fig. 1) demonstrates that there is significant variability (P=0.008) in airborne bacterial counts across the 10-h sampling period in all 3 independent case studies conducted in patient-occupied isolation room studies regardless of patient scenario (Table I).

Airborne bioburden monitoring over 24-h patient-occupied isolation rooms

The first 24-hour case study was conducted in a room occupied for 10 days by a 70-year-old female with respiratory failure on a background of gastroenteritis and *Clostridium difficile* infection (Fig. 2a). Over the 24-hour period, the mean air bioburden was 104.4±96.2 cfu/m^3 with minimum and maximum recorded values of 12 and 510 cfu/m^3, respectively. When the dataset was divided into 'day' and 'night' (08:00–20:00h and 20:00–08:00h, respectively), the mean airborne count from the 'day' was 151.2 cfu/m^3, in comparison to a mean 'night' value of 56.6 cfu/m^3 (P<0.001). The 'out of control' levels collected at 11:15–11:45h (observations 14-16) were a direct result of a high degree of room activity in which an increased staff presence (from 1 to 5) aided the movement of the patient from a bed via a mechanical hoist. Additionally, the footfall in and out of the room was substantially higher during these samples leading to a peak count of 510 cfu/m^3, the highest level of air bioburden recorded across the entire set of case studies.

Figure 2b displays the air monitoring results in a room occupied for 6-days by a male patient with Guillian-Barre demyelinating disease and widespread muscle weakness. Air contamination levels varied substantially across 24 hours, with 'out of control' levels occurring during visiting hours. The mean value across the 24 hours was 102.4±68.8 cfu/m^3 with a minimum value of 5 cfu/m^3 recorded at 04:45h and maximum value of 355 cfu/m^3 recorded at 14:45h. The mean values for 'day' and 'night' were 113.6 and 91.0 cfu/m^3 respectively (P=0.080), (Table I).

The final case study (Fig. 2c) was conducted in an isolation room occupied for 1 day by a 56-year-old immunocompromised female patient with respiratory failure and background of rheumatoid arthritis. The overall mean value across the 24-hour case study was 62.1±82.4 cfu/m^3 with a range of 0–398 cfu/m^3. An initial surge in airborne bacteria to the maximum value of 398 cfu/m^3 occurred in response to an increase in staff presence required to assist patient intubation. Significantly high levels of 214 cfu/m^3 were also observed when a ventilator was changed (Observation 9; 10:00h). Contamination

levels peaked again at observation 33-36 (16:00–17:00h), during which the patient was wheeled out of the room for a CT scan resulting in air counts of 300–400 cfu/m^3. The mean day time value was 86.9 cfu/m^3, followed by relatively low and consistent values during the night with an average of 36.7 cfu/m^3 (P=0.002). Counts then increased from observation 94–97 (07:15-08:00h) during morning handover.

As a baseline control for comparison, monitoring was also conducted in an empty isolation room (Fig. 2d). Airborne bacteria levels were low and consistent across the 24 hours, however average values between 'day' and 'night' still varied from 26.8 cfu/m^3 (08:00–20:00h) to 13.0 cfu/m^3 (20:00—8:00h) (P<0.001). An overall mean value of 20.0±14.2 cfu/m^3 was recorded. Significant ('out of control') levels occurred within this dataset during cleaning of the empty room.

Correlation of high air bioburden levels to room activity

Table II details specific room activities which were consistently linked to high levels of air contamination across all studies, based on the collated activity logs. Increases in air bioburden as a result of each activity were calculated as a percentage increase from the sample mean of the corresponding study to allow a fair comparison. The two 'highest risk' activities for increasing bioburden were (i) the movement/operation of large pieces of equipment and (ii) an increased number of staff in the room. The movement or operation of large equipment into and around patient rooms (e.g. x-ray scanners, mechanical hoists, trolleys) resulted in an increase in air bioburden of 197.6%, with a range of 3.1-540.9% (n=16). An increase in staff numbers within patient rooms caused similar peaks in contamination levels. When >3 staff were present in the room, air counts increased by an average of 197.1% (n=15) from the sample mean. Percentage increase values ranged from 18.2-518.4%. When this scope was widened to include staff numbers greater than 2, the average increase in airborne bacteria was 154.7% (n=43), with a range of 1.5-540.9%. The highest recorded number of staff in a patient isolation room at a given time across all case studies was 9. Other 'high risk'

activities included bed changes (+145.3%), patient personal hygiene/turn (+103.9%), visiting hours (+83.8%) and cleaning (+56.6%).

Discussion

Understanding the route and transmission of infectious microorganisms plays a key role in infection prevention. Recently, the role of the environment as a source of infection within clinical establishments has been increasingly documented [15]. However, to date, there have been few studies which have characterised levels of airborne microorganisms within an ICU over extended time periods. Previous clinical air studies have focused on short time periods or specific activities of interest [14, 16, 17]. The present study has significantly expanded this information by successfully demonstrating the levels and fluctuations of airborne bacteria within an ICU during different patient and environmental scenarios over 10 and 24-hour periods.

Airborne microbial counts were shown to greatly vary across the 10-h or 24-h sampling periods during all case studies (Table I), and this variation was expected given the extremely dynamic nature of an ICU. Results also enabled peaks in airborne bacterial load to be correlated to specific activities, and particular activities to be statistically classified as 'out-of-control', but it is important to bear in mind that these 'out-of-control' peaks are relative only to the dataset as a whole in which they were recorded.

Mean bioburden levels recorded in this study are lower than those from other ICU studies which have reported levels between 350–450 cfu/m^3 [18, 19], and higher than those from a more recent study (<40 cfu/m^3) [20]. The differences are likely due to confounding factors including differences in air change ventilation rates, number of medical staff and patients, patient conditions, and importantly, the sample number and collection times. The degree of variation evidenced in the different case studies in

the present work demonstrates that mean levels will be significantly different if different sampling periods and/or lower sample numbers are used.

Extensive variation in air counts was observed in 10-hour patient-occupied isolation room studies, and mean values reflected the length of room occupation, with one exception (Fig. 2b). In this study the patient occupied the room for 7 days, but the mean airborne bacterial load was only 44.1 cfu/m^3. This correlated well with room activity, as in this case, the patient was conscious and required little 1:1 care.

Results from 24-hour monitoring also indicated that the longer the patient occupied the room, the greater the mean cfu/m^3, and additionally, the mean 'day' airborne counts were statistically different to the equivalent 'night' levels (P<0.001). This observation reflected the reduced activity in the unit overnight. However, it was interesting to observe that a patient turn activity, which resulted in a significant peak in air counts during the day time (Fig. 3c, observation numbers 34 and 75), had minimal effects when carried out during the night. This potentially indicates that the activity of the unit as a whole contributes to air counts even within individual isolation rooms, highlighting how easily airborne microorganisms are dispersed through the ICU in general. Studies in Burns units have demonstrated the ease by which bacteria are liberated from the patient into the air [21]. One study showed that 31% of dressing changes on MRSA positive burns patients liberated the organism into the air [22]. A similar finding was observed in the present study, whereby an average increase in cfu/m^3 of 103.9% (n=16) was recorded during patient personal hygiene/turn activities involving bed bathing and physical movement of the patient.

A number of patient care-related activities contributed to peaks in air contamination levels, most of which are centred on an increase in people traffic. It is estimated that each individual disperses

approximately 10^4 particles while walking, many of which are viable and some pathogenic, meaning the more people present in a room, the greater the chance of dispersing biological particles which may have the potential to cause harm [23, 24]. This is relevant to the present study where an average percentage increase in air bioburden of 197.1% was generated as a result of >3 staff members present in the isolation room. Bed sheet changes have also been implicated in the increase in aerial dispersal of bacteria. In the present study, this caused an average increase of 145.3% (n=7). Previous studies have recorded similar results whereby mean counts of airborne MRSA from infected patients increased from 4.7 cfu/m^3 to 116 cfu/m^3 during bed sheet changes and remained elevated for some time after the event [25]. Similarly, air counts of up to 2614 cfu/m^3 were recorded in response to bed changes in a Burns Unit, with elevated levels persisting for up to 60 minutes [14].

A previous study monitored variations in airborne bioaerosols in a hospital ward in response to general ward activities, however the longest period of air sampling was 8-hours, with no account of overnight activity and air data [16]. Results agree with the present study in terms of bioaerosol-generating activities and increased dispersal during early mornings when ward activity was high. A strong correlation between increased viable counts and increased *Staphylococcus* species was also observed, indicating the likelihood of an increased dispersal of *S. aureus* when peaks in air contamination occurred. Most 'high risk' activities identified have been previously linked to high airborne bacterial levels, with one exception. The movement of large medical equipment into/within patient rooms caused the highest overall average increase in air bioburden at 197.6% (n=16). This could be due to movement of large air volumes already containing viable organisms or may implicate equipment as significant environmental reservoirs of microorganisms within the ICU.

Surfaces have been well implicated in the cross-infection of patients by acting as reservoirs for the transmission of microorganisms, but there still remains uncertainty regarding the degree of contribution of the airborne route to the overall spread of infection. However, pneumonia and

respiratory tract infections were the second largest group of HAIs and accounted for 22.4% of the total HAIs in Scotland in 2016 [26]. All airborne microbes ultimately end up depositing onto surrounding surfaces, and so can indirectly contribute to infection transmission via direct surface contact. A recent study aimed to establish a correlation between air and surface microbes in the critical care environment, further emphasising this phenomenon [20]. Their research found a strong association between passive air sampling counts and surface counts and indeed made the important point that surface bacteria will include a portion of airborne bacteria after settling. Settle plate standards were also proposed in 2000, as an 'Index of microbial air contamination (IMA)', a passive form of air sampling in which microbial contamination from the air is evaluated after it has settled onto the surface of agar plates [27]. Using settle plates as part of routine environmental screening for HAI risk from airborne contaminants could be a positive addition to infection control strategies, however, as shown in the present study through active air sampling, biologically active particles are present at all times in the air of the ICU, even in unoccupied rooms. Therefore, if using passive sampling methods, care should be taken to ensure that counts are not underestimated due to the potential for droplet nuclei to remain suspended for prolonged periods [6].

As a limitation, identification of the collected microorganisms was not possible. It is important to note though, that although certain activities resulted in high levels of air bioburden, this does not necessarily correlate to a high level of pathogenic organisms. Recently, it was shown that environmental bioburden measured by total colony count did not predict the presence of clinically relevant pathogenic organisms [28]. Additionally, viral collection was not possible with this methodology. Future consideration should be given to identification and correlation of airborne microorganisms with strains originating from the patients housed in the environment, however for the present study the scope was to assess overall variability of airborne bacteria and changes in response to key activities.

Conclusion

This study successfully recorded for the first time, environmental air contamination levels in an ICU across 24-hour time periods. Bioaerosol counts varied significantly across sampling periods, however peaks were a direct result of room activity, in particular during the presence of increased numbers of medical staff and/or use of large equipment. Various other factors contributed to increased levels of air contamination, predominantly length of room occupation and people traffic. Although these results are specific to this ICU setting, this study provides an insight into the typical background levels of airborne microorganisms in the critical care setting, and how they change in response to the everyday operation of this dynamic environment. A greater understanding of the airborne transmission route and the clinical airborne microflora is required to more fully understand the role of airborne pathogens in the spread of HAIs, with the aim of establishing more direct and continuous infection control strategies.

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Conflict of Interest Statement

None declared.

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Figure Captions

Fig 1. Statistical Control Charts (Minitab v17) demonstrating levels of airborne bacteria over a 10-hour period (08:00-18:00) in patient-occupied isolation rooms within an ICU. Rooms were occupied by patients for differing periods prior to the commencement of air sampling: (a) 8 days, (b) 7 days, and (c) 3 days. Each data point represents the probable cfu/m^3 from air samples taken at 15-minute intervals and incubated for 48 hours. 'Out of control' data points are highlighted in red. 'High risk' activities leading to increased airborne bioburden above the mean are identified as follows: a=increase in staff presence >3; b=patient personal hygiene/turn; c=bed/sheet changes; d=visiting; e=movement of large equipment into/around room; f=cleaning. (n=41; UCL = upper control limit; $\overline{X}$ = mean; LCL = lower control limit).

Fig 2. Statistical Control Charts (Minitab v17) demonstrating levels of airborne bacteria over a 24-hour period (08:00-08:00) in occupied and unoccupied inpatient isolation rooms of an ICU. In patient occupied rooms, rooms were occupied by patients for differing periods prior to the commencement of air sampling: (a) 10 days, (b) 6 days, and (c) 1 day. Monitoring of an empty patient room was also included for comparison (d). For analysis, periods of 'Day' and 'Night' were categorised as 08:00-20:00 and 20:00-08:00, respectively. Each data point represents the probable cfu/m^3 from air samples taken at 15-minute intervals and incubated for 48 hours. 'Out of control' data points are highlighted in red. 'High risk' activities leading to increased airborne bioburden above the mean are identified as follows: a=increase in staff presence >3; b=patient personal hygiene/turn; c=bed/sheet changes; d=visiting; e=movement of large equipment into/around room; f=cleaning. (n=97; UCL = upper control limit; $\overline{X}$ = mean; LCL = lower control limit).

Table I. Summary of data generated from the different case studies within an ICU monitoring the microbial air contamination levels across 10- and 24-hour sampling periods. Data were recorded in occupied and empty patient isolation rooms. For each study, details are also provided for the ward activities which were associated with the significant increases in airborne bioburden (the 'out of control' observations, as highlighted by the statistical process control charts (Figs 1-3)). Mean and standard deviation were recorded for each 10 hour case study (n=46), whilst 24 hour studies were further analysed via day (08:00 – 20:00) and night (20:00 – 08:00) portions of the sample collection period (n=97).

Case Study (Figure)	Length of Room Occupancy (days)	Total Mean (cfu/m^3 ±SD)	Total Range (cfu/m^3)	P Value (95% C.I)	Mean Day (08:00-20:00) (cfu/m^3 ±SD)	Mean Night (20:00 – 08:00) (cfu/m^3 ±SD)	P Value for Day v. Night (95% C.I)	Activities which contributed to increased bioburden and consequent failing of control chart statistical tests (Observation No.)
INPATIENT ISOLATION ROOM 10 HOUR STUDIES								
Fig. 1a	8	64.3 (±31.8)	12-166	0.008	-	-		Fresh bed sheets shaken (16)
Fig. 1b	7	44.2 (±36.1)	8-166		-	-		Increased staff presence from 0 to 2 (9)
Fig. 1c	3	48.8 (±20.5)	20-116		-	-		Patient helped out of bed (32-33) Bed removed from room (35)
INPATIENT ISOLATION ROOM 24 HOUR STUDIES								
Fig. 2a	10	104.4 (±96.2)	12-510	<0.001	151.2 (±111.9)	56.6 (±39.1)	<0.001	Patient turn, patient physio, operation of mechanical hoist, high staff presence (14-18) Increased people traffic from 1 (visitor) to 2 (visitor + nurse) (33)
Fig. 2b	6	102.4 (±68.8)	5-355		113.6 (±79.4)	90.9 (±54.3)	0.080	Increased people traffic from 0 to 2 (visitor + nurse) (27-29)
Fig. 2c	1	62.1 (±82.4)	0-398		86.9 (±95.8)	36.7 (±56.3)	0.002	Increased staff presence from 2-5 staff (4) Ventilator change (9) Patient in bed taken for CT scan followed by return (33,34) Patient turn (36)
Fig. 2d	0	20.0 (±14.2)	2-90		26.8 (±16.3)	13.0 (±6.6)	<0.001	Room cleaning (17) Brief open and close of door (31) Handover of sampler (49)

Table II. Overview of the 'high risk' ward activities which contributed to increases in airborne microbial bioburden. Activities which consistently correlated to high air counts were selected, and percentage increases in cfu/m^3 were calculated from the sample mean of the corresponding case study. The overall average percentage increase is given, alongside the sample size (n).

Activity	Average % increase from sample mean	Range (%)
Increase in staff presence >3	197.1	18.2-518.4 (n=15)
Personal patient hygiene/turn	103.9	1.5-359.8 (n=16)
Bed/sheet changes	145.3	1.5-276.4 (n=7)
Visiting	83.8	5.4-247.3 (n=23)
Movement of large equipment into/around room	197.6	3.1-540.9 (n=16)
Cleaning	56.6	27.1-95.4 (n=5)

Figure 1(a)

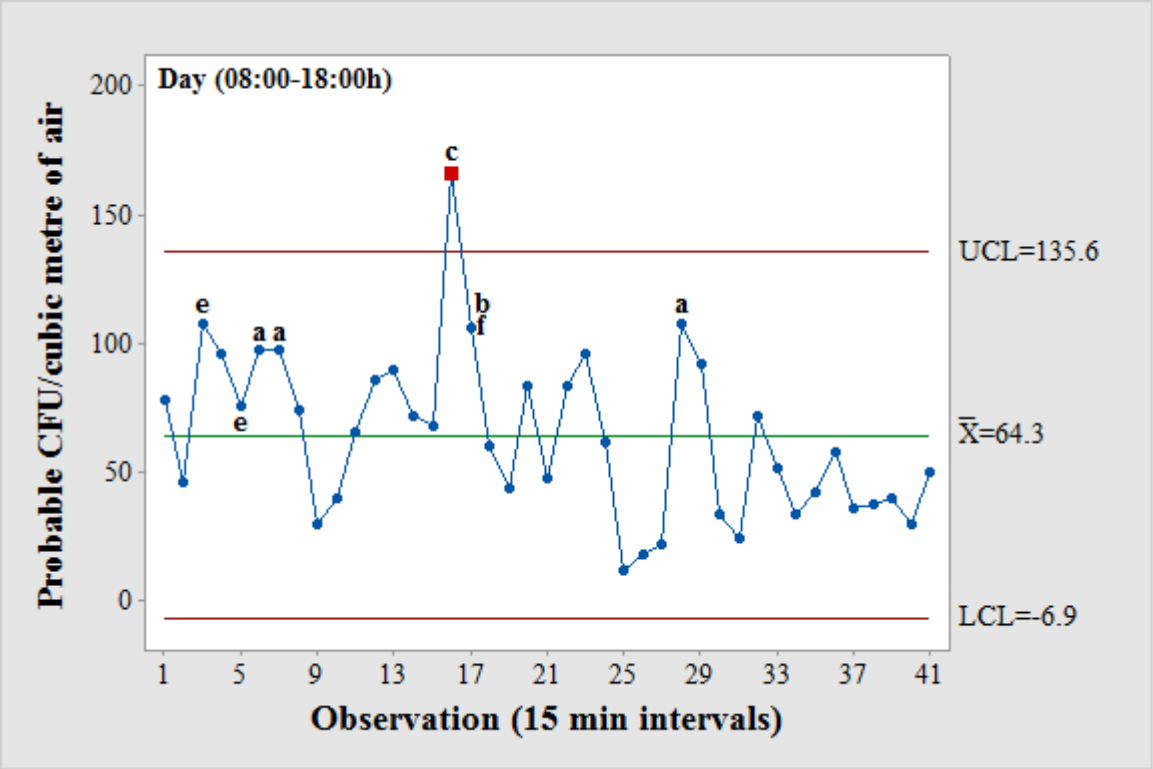


Figure 1(b)

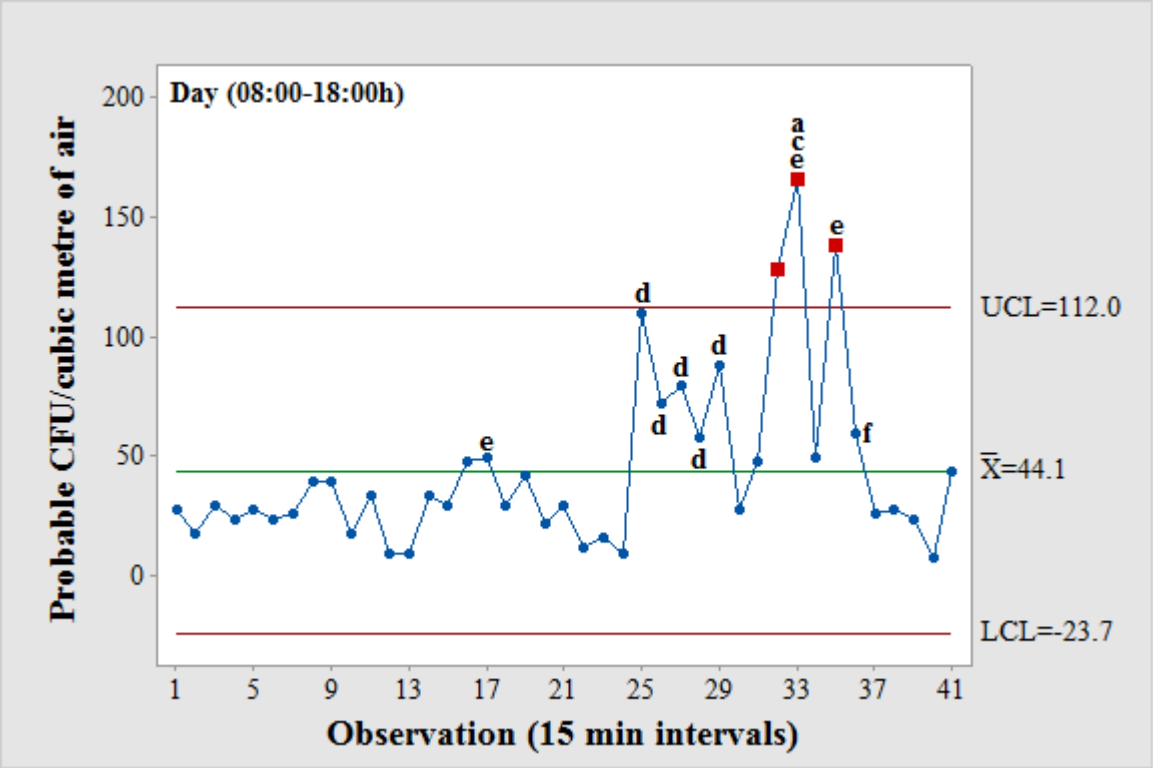


Figure 1(c)

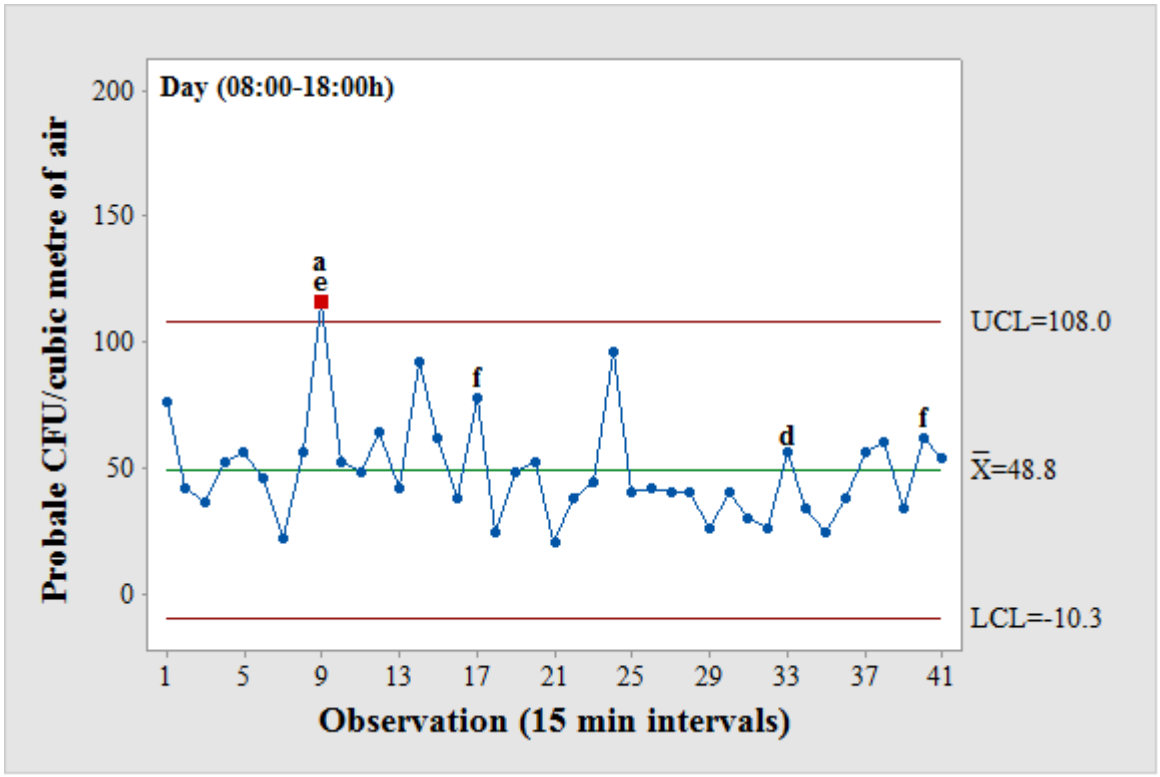


Figure 2(a)

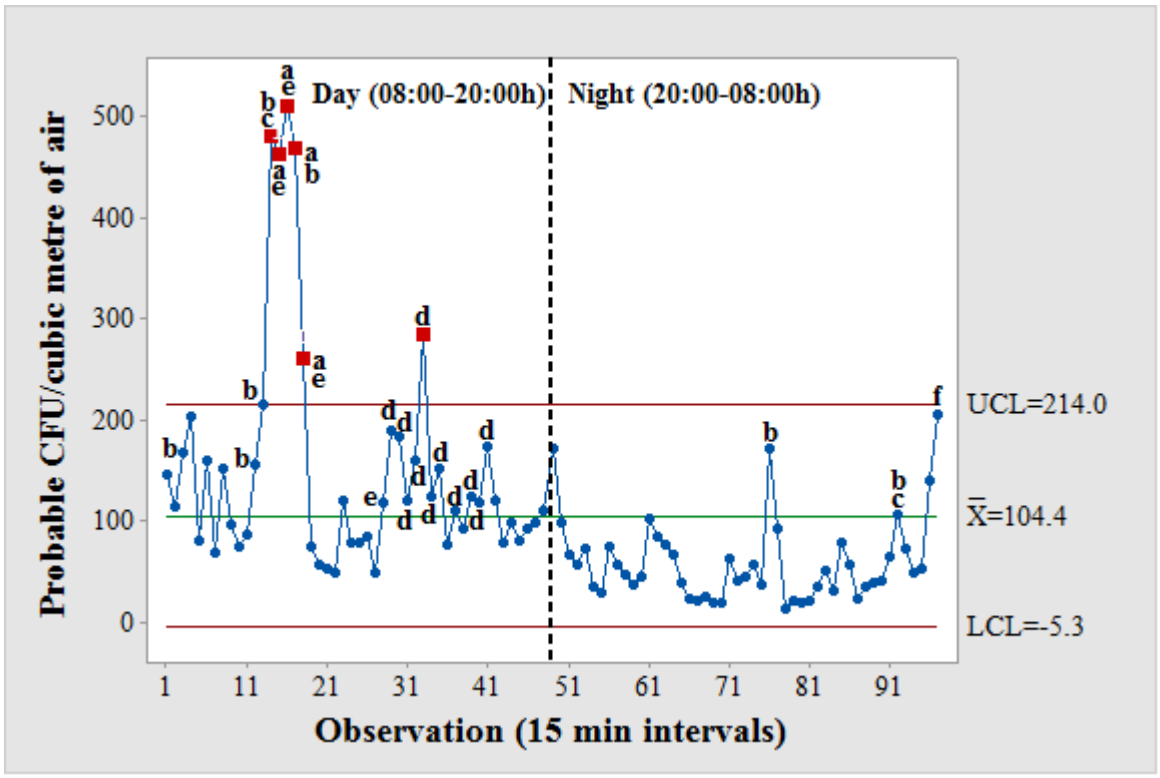


Figure 2(b)

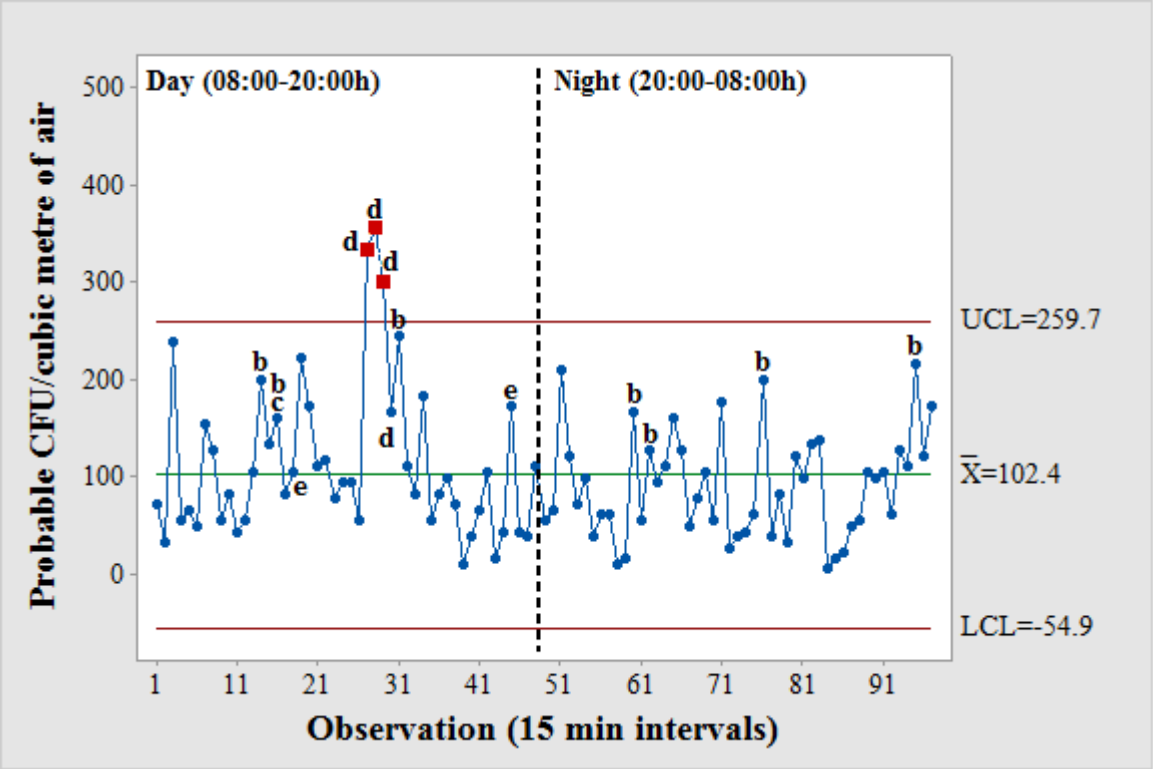


Figure 2(c)

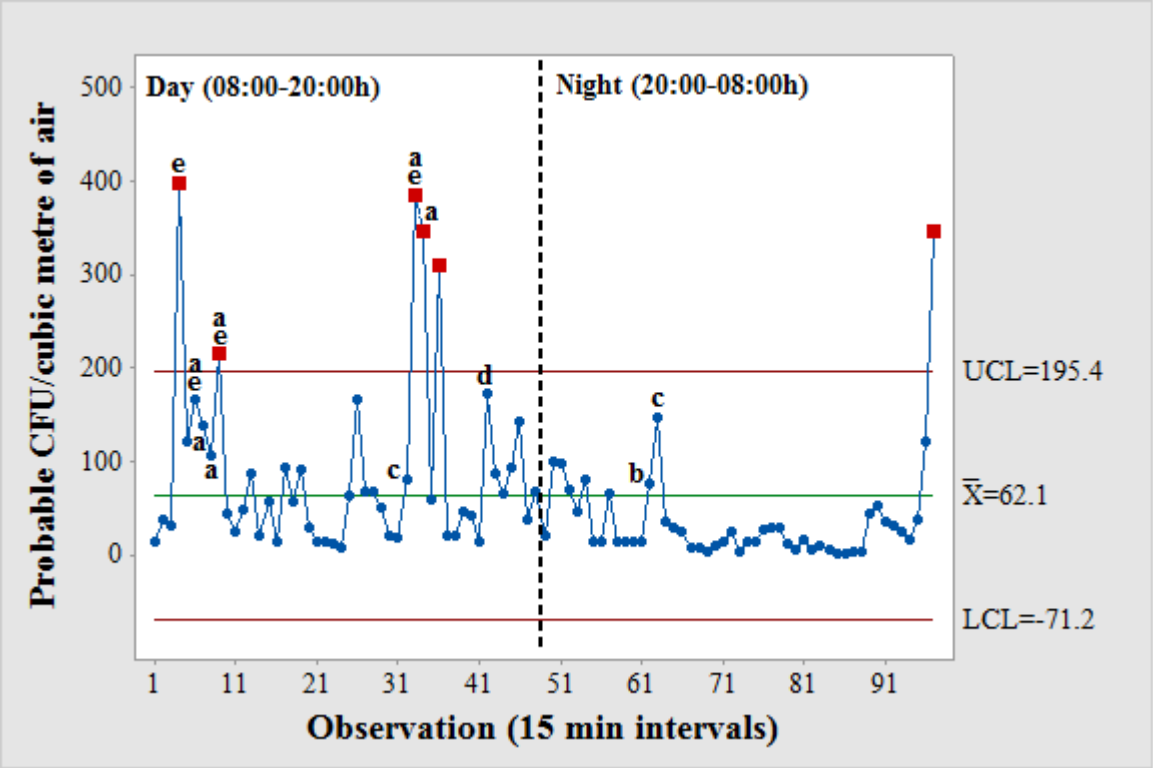
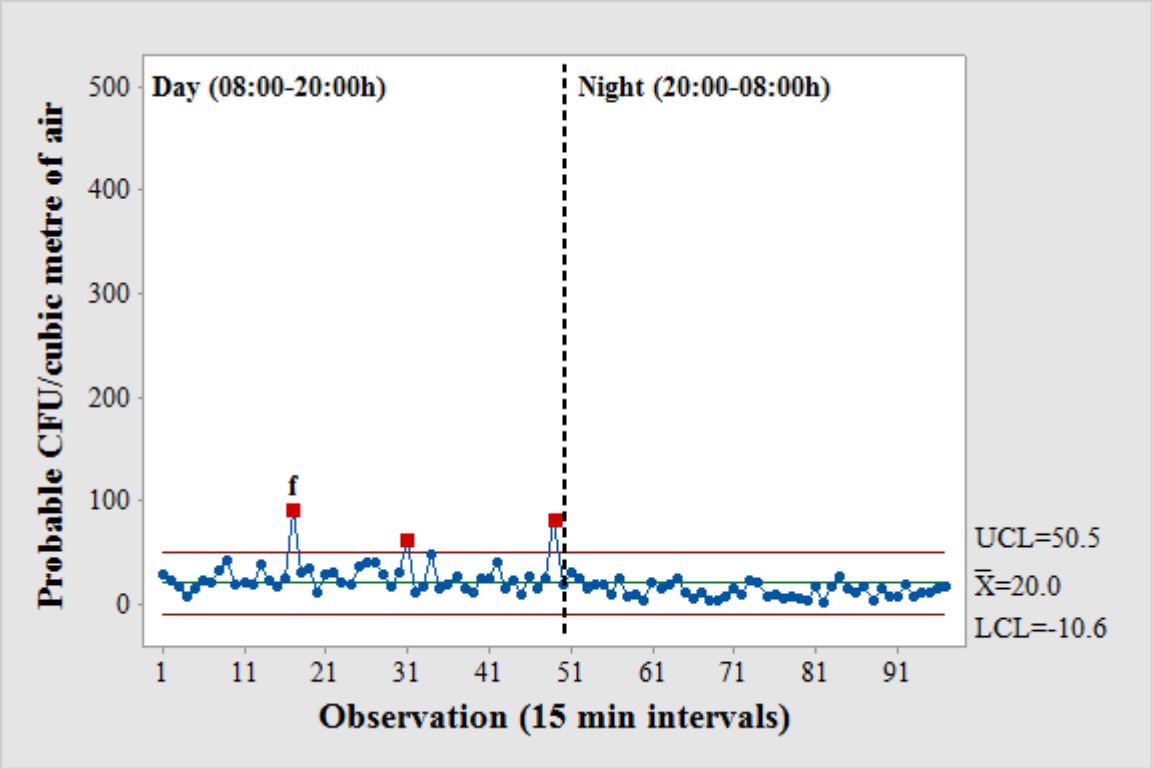


Figure 2(d)


Day (08:00-20:00h)
Night (20:00-08:00h)
Probable CFU/cubic metre of air
500
400
300
200
100
0
f
UCL=50.5
X̄=20.0
LCL=-10.6
1
11
21
31
41
51
61
71
81
91
Observation (15 min intervals)