COMPARISON OF PARTICLE COUNTING AND MICROBIOLOGICAL SAMPLING METHODS AT-REST AND DURING SURGERY

by

Ahmet Emir Kavak

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ABSTRACT

COMPARISON OF PARTICLE COUNTING AND MICROBIOLOGICAL SAMPLING METHODS AT-REST AND DURING SURGERY

Air quality monitoring in operating rooms is of prime importance because particles that carry microbiological contamination generate serious risks during surgical operations. Air quality monitoring is executed by two separate methods: Microbiological sampling and particle counting.

The aim in this project is to investigate statistical correlation between these two methods. If so, particle counting technique could replace frequent microbiological sampling. Microbiological sampling and particle counting techniques are applied in five operating rooms located at three different hospitals with a total number of 360 measurements taken before (at rest) and during surgery. Spearman's correlation coefficient is used for measuring the level of correlation between two methods. The bacteria counts are classified after their sizes and tested for size-by-size correlation. Then, the bacterial counts measured at the same site are cumulatively added together and correlated with the particle counts at each particle size-range.

No correlation is found when size-by-size correlation is performed. When cumulative bacteria counts are considered in 'at-rest' conditions, the number of particles in 5.0-10.0 μm and 10.0-25.0 μm size ranges correlated with bacteria counts. Particles of 1.0-5.0 μm size ranges and particles larger than 25.0 μm correlated with bacteria counts during surgery.

Keywords: Particle Counting, Microbiological Sampling, Air Quality Monitoring, Operating Rooms, Surgery.

ÖZET

PARTİKÜL SAYIMI VE MİKROBİYOLOJİK ÖRNEKLEME TEKNİKLERİNİN AMELİYET ÖNCESİ VE AMELİYAT SIRASINDAKİ ÖLÇÜMLERLE KARŞILAŞTIRILMASI

Ameliyathanelerdeki hava kalitesinin izlenmesi, mikrobiyolojik kirlilik taşıyan parçacıkların yol açacağı risklerin ciddi olmasından dolayı birincil öneme sahiptir. Hava kalitesinin izlenmesi için iki metot bulunmaktadır: Mikrobiyolojik örnekleme ve partikül sayımı.

Bu projenin amacı, bu iki metot arasında istatistiksel bir ilişki bulmaya çalışmaktır. Eğer varsa, partikül sayımı, sık yapılan mikrobiyolojik örneklemelerin yerini alabilecektir. Her iki metot kullanılarak üç farklı hastanede bulunan toplam beş ameliyathanede ameliyat öncesi ve ameliyat sırasında toplam 360 ölçüm alınmıştır. Aradaki korelasyonun hesaplanmasında Spearman'in Korelasyon Katsayısı kullanılmıştır. Bakteri miktarı, boyutlarına göre sınıflandırılarak boyut eksenli korelasyona bakılmıştır. Daha sonra, aynı mevkideki bakteri miktarları toplanarak partikül boyutları ile korelasyonuna bakılmıştır.

Boyut eksenli kıyaslamada korelasyon bulunamamıştır. Kümülatif bakteri miktarının partikül boyutlarıyla korelasyonunda ise; ameliyat öncesi ölçümlerde 5.0-10.0 μm ve 10.0-25.0 μm boyut aralığındaki partiküllerle bakteri miktarı arasında korelasyon saptanmıştır. Ameliyat esnasındaki ölçümlerde ise 1.0-5.0 μm boyut aralığında ve 25.0 μm'den büyük olan partiküller ile bakteri miktarı arasında korelasyon saptanmıştır.

Anahtar Sözcükler: Partikül Sayımı, Mikrobiyolojik Örnekleme, Hava Kalitesi İzlenmesi, Ameliyathane, Ameliyat

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R^2	Coefficient of Determination
h	Result of Hypothesis Test
p	Probability of Obtaining a Test Statistic
ks	Result of Kolmogorov-Smirnov Test

LIST OF ABBREVIATIONS

MMAD	Mass Median Aerodynamic Diameter
CMAD	Count Median Aerodynamic Diameter
HEPA	High Efficiency Particulate Air
PCR	Polymerase Chain Reaction
PE	Protective Environment
SSI	Surgical Site Infection
UVGI	Ultraviolet Germicidal Irradiation
UV	Ultraviolet
HVAC	Heating, ventilation, and air conditioning
NFPA	National Fire Protection Association
ISO	International Organization for Standardization
ANSI	American National Standards Institute
IEST	Institute of Environmental Sciences and Technology
RSV	Respiratory Syncytial Virus
VZV	Varicella Zoster Virus
PSL	Polystyrene Latex
CFM	Cubic Foot Per Minute
SDA	Sabouraud's Dextrose Agar
ACFM	Actual Cubic Feet Per Minute
RHO	Spearman's rank correlation coefficient

1. INTRODUCTION

1.1 Background and Motivation

The healthcare environment contains a diverse population of microorganisms, but only a few are significant pathogens for susceptible humans. Microorganisms are present in great numbers in moist, organic environments, but some also can persist under dry conditions. Although pathogenic microorganisms can be detected in air and water and on fomites, assessing their role in causing infection and disease is difficult [4]. Only a few reports clearly delineate a cause and effect with respect to the environment and in particular, housekeeping surfaces.

Air sampling is used to detect aerosols (i.e., particles or microorganisms). Particulate sampling (i.e.,total numbers and size range of particulates) is a practical method for evaluating the infection-control performance of operating rooms, with an emphasis on filter efficiency in removing respirable particles (<5 μm in diameter) or larger particles from the ambient air. Particle size is reported in terms of the mass median aerodynamic diameter (MMAD), whereas count median aerodynamic diameter (CMAD) is useful with respect to particle concentrations [1].

Particle counts in a given air space within the healthcare facility should be evaluated against counts obtained in a comparison area. Particle counts indoors are commonly compared with the particulate levels of the outdoor air. This approach determines the rank order air quality from dirty (i.e., the outdoor air) to clean (i.e., air filtered through high-efficiency filters [90%-95% filtration]) to cleanest (i.e., HEPA-filtered air) [5]. Comparisons from one indoor area to another may also provide useful information about the magnitude of an indoor air-quality problem. Making rank-order comparisons between clean, highly-filtered areas and dirty areas and/or outdoors is one way to interpret sampling results in the absence of air quality and action level standards [6]-[7].

Particle counters and anemometers are used in particulate evaluation. The anemometer measures air flow velocity, which can be used to determine sample volumes. Particulate sampling usually does not require microbiology laboratory services for the reporting of results [1]. Also, particle counting is less demanding and offers immediate results [8].

Microbiologic sampling of air in healthcare facilities remains controversial because of currently unresolved technical limitations and the need for substantial laboratory support. The unresolved issues associated with microbiologic air sampling are:

1. Lack of standards linking fungal spore levels with infection rates (i.e., no safe level of exposure),
2. Lack of standard protocols for testing (e.g., sampling intervals, number of samples, sampling locations),
3. Need for substantial laboratory support,
4. Culture issues (e.g., false negatives, insensitivity, lag time between sampling and recording the results),
5. New, complex polymerase chain reaction (PCR) analytical methods, Unknown incubation period for Aspergillus spp. Infection,
6. Variability of sampler readings,
7. Sensitivity of the sampler used (i.e., the volumes of air sampled),
8. Lack of details in the literature about describing sampling circumstances (e.g., unoccupied rooms vs. ongoing activities in rooms, expected fungal concentrations, and rate of outdoor air penetration),
9. Lack of correlation between fungal species and strains from the environment and clinical specimens,
10. Confounding variables with high-risk patients (e.g., visitors and time spent outside of protective environment [PE] without respiratory protection),

11. Need for determination of ideal temperature for incubating fungal cultures (95°F [35°C] is the most commonly used temperature) [6]-[7]-[9]-[10]-[11]

Sedimentation methods using settle plates and volumetric sampling methods using solid impactors are commonly employed when sampling air, for bacteria and fungi. Settle plates have been used by numerous investigators to detect airborne bacteria or to measure air quality during medical procedures (e.g., surgery) [12]. Settle plates, because they rely on gravity during sampling, tend to select for larger particles and lack sensitivity for respirable particles (e.g., individual fungal spores), especially in highly-filtered environments. Therefore, they are considered impractical for general use [6]. Settle plates, however, may detect fungi aerosolized during medical procedures (e.g., during wound dressing changes), as described in a recent outbreak of aspergillosis among liver transplant patients [13].

The use of slit or sieve impactor samplers capable of collecting large volumes of air in short periods of time are needed to detect low numbers of aerosols in highly filtered areas [6]-[7].

Air sampling in healthcare facilities, whether used to monitor air quality during construction, to verify filter efficiency, or to commission new space prior to occupancy, requires careful notation of the circumstances of sampling. Most air sampling is performed under undisturbed conditions. However, when the air is sampled during or after human activity (e.g., walking and vacuuming), a higher number of airborne microorganisms likely is detected [11]. The contribution of human activity to the significance of air sampling and its impact on healthcare-associated infection rates remain to be defined.

1.2 Objectives

Main objective of this study is to examine the relationship between the two methods in air quality monitoring of operating theatres. The relationship between these two methods has rarely been evaluated in operating theatres and a significant correlation between these methods are not found [8]-[14]. In this project, New-Star Six-Stage Andersen Viable Sampler is used for microbiological sampling that can range the size of the viable particles like particle counters do. The goal of this study is to classify the degree and size of microbiological contamination of the air and non-viable particles in operating rooms, and to look whether there is a size-by-size correlation between the two methods which is not examined previously.

1.3 Outline of the thesis

Chapter 1 introduces the subject, presents the motivation of the thesis and gives an outline. Chapter 2 gives a brief information about the operating rooms and modes of transmission of airborne diseases. In Chapter 3, the air quality monitoring techniques in operating rooms are introduced. Experimental procedure and measurement methodology is described in Chapter 4. In Chapter 5, results are given. In chapter 6, data analysis and statistical analysis will be presented with a discussion. Finally, a general conclusion will be made in Chapter 7.

2. OPERATING ROOMS AND MODES OF TRANSMISSION OF AIRBORNE DISEASES

2.1 Operating Rooms

Operating room air may contain microorganisms, dust, aerosol, lint, skin squamous epithelial cells, and respiratory droplets. The microbial level in operating room air is directly proportional to the number of people moving in the room [15]. One study documented lower infection rates with coagulase-negative staphylococci among patients when operating room traffic during the surgical procedure was limited [16]. Therefore, efforts should be made to minimize personnel traffic during operations. Outbreaks of SSIs caused by group A beta hemolytic streptococci have been traced to airborne transmission from colonized operating room personnel to patients [17]-[18]. Several potential healthcare-associated pathogens (e.g., Staphylococcus aureus and Staphylococcus epidermidis) and drug-resistant organisms have also been recovered from areas adjacent to the surgical field [19], but the extent to which the presence of bacteria near the surgical field influences the development of postoperative SSIs is not clear [20].

Proper ventilation, humidity ($<$68%), and temperature control in the operating room is important for the comfort of surgical personnel and patients, but also in preventing environmental conditions that encourage growth and transmission of microorganisms [21]. Operating rooms should be maintained at positive pressure with respect to corridors and adjacent areas [22].

Laminar airflow and UVGI have been suggested as adjunct measures to reduce SSI risk for certain operations. Laminar airflow is designed to move particle-free air over the aseptic operating field at a uniform velocity (0.3-0.5 m/sec), sweeping away particles in its path. This air flow can be directed vertically or horizontally, and recirculated air is passed through a HEPA filter [23]. Neither laminar airflow nor UV light, however, has been conclusively shown to decrease overall SSI risk [24].

2.1.1 Heating, Ventilation, and Air Conditioning Systems in Healthcare Facilities

Heating, ventilation, and air conditioning (HVAC) systems in healthcare facilities are designed to a) maintain the indoor air temperature and humidity at comfortable levels for staff, patients, and visitors; b) control odors; c) remove contaminated air; d) facilitate air-handling requirements to protect susceptible staff and patients from airborne healthcare-associated pathogens; and e) minimize the risk for transmission of airborne pathogens from infected patients [6]-[25]. An HVAC system includes an outside air inlet or intake; filters; humidity modification mechanisms (i.e., humidity control in summer, humidification in winter); heating and cooling equipment; fans; ductwork; air exhaust or out-takes; and registers, diffusers, or grilles for proper distribution of the air (Figure 2.1) [26]. Decreased performance of healthcare facility HVAC systems, filter inefficiencies, improper installation, and poor maintenance can contribute to the spread of healthcare-associated airborne infections.

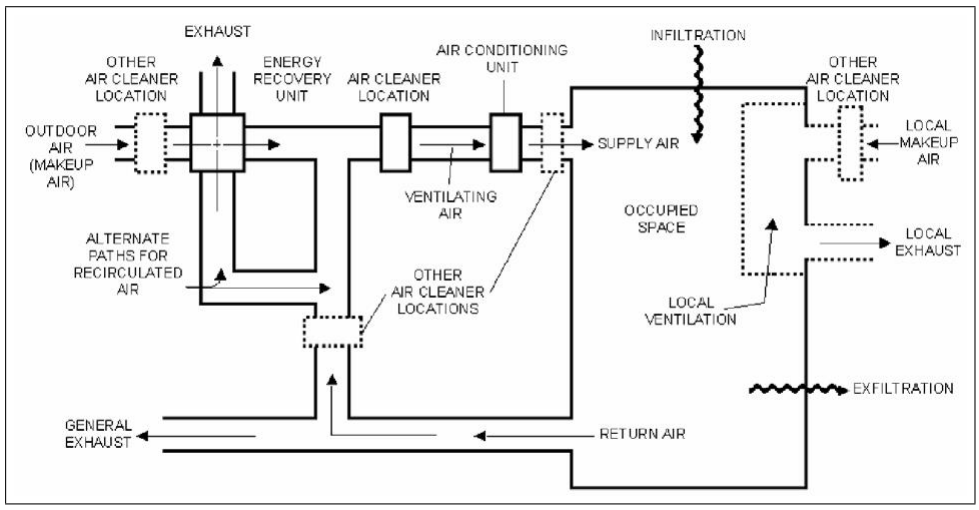

Figure 2.1 Diagram of a ventilation system in a hospital.[1]

The following design conditions are recommended for operating, catheterization, cystoscopic, and fracture rooms :

1. There should be a variable range temperature capability of 20 °C to 24°C.

2. Relative humidity should be kept between 50% and 60%.
3. Positive air pressure should be maintained by supplying about 15% excess air.
4. Differential pressure indicating device should be installed.
5. Humidity indicator and thermometers should be located for easy observation.
6. Filter efficiencies should be in accordance with codes.
7. Entire installation should conform to NFPA Standard 99, Health Care facilities.
8. All air should be supplied at the ceiling and exhausted from at least two locations near the floor.
9. Control centres that monitor and permit adjustment of temperature, humidity, and air pressure may be located at the surgical supervisor's desk. [27]

2.1.2 Classification of Operating Rooms

The US Federal Standard 209E, published in 1963, defined cleanroom classification and monitoring within the United States. The European Committee for Standardization, in cooperation with the International Organization for Standardization (ISO), developed standards for Europe. Different standards caused confusion, so in 1992, the American National Standards Institute (ANSI) and Institute of Environmental Sciences and Technology (IEST) petitioned ISO to develop an international standard.

ISO developed new standards for cleanroom classifications and monitoring and published them under ISO 14644. In November 2001, the United States adopted ISO 14644 standards and officially cancelled FS-209E. Table 2.1 compares cleanroom classifications for FS-209E and ISO 14644-1.

ISO 14644-1 establishes standard classes of air cleanliness for cleanrooms and clean zones based on specified concentrations of airborne particulates. An ISO Class 1 cleanroom has no more than 10 particles larger than 0.1 μm in any given cubic meter

Table 2.1
Equivalence of FS209E and ISO.

FS-209E (Particles/(ft^3))	ISO 14644-1
	1
	2
1	3
10	4
100	5
1000	6
10000	7
100000	8
	9

of air. An ISO Class 2 cleanroom would be ten times dirtier than a Class 1 cleanroom, and an ISO Class 3 cleanroom would be ten times dirtier than a Class 2, and so forth. The specific allowable particle limits per ISO Class are shown in Figure 2.2 [28].

Classification numbers Numbers (N)	Maximum concentration limits (particles/m^3 of air) for particles equal to and larger than the considered sizes shown below					
	0.1µ m	0.2µ m	0.3µ m	0.5µ m	1µ m	5.0µ m
ISO 1	10	2				
ISO 2	100	24	10	4		
ISO 3	1 000	237	102	35	8	
ISO 4	10 000	2 370	1 020	352	83	
ISO 5	100 000	23 700	10 200	3 520	832	29
ISO 6	1 000 000	237 000	102 000	35 200	8 320	293
ISO 7				352 000	83 200	2 930
ISO 8				3 520 000	832 000	29 300
ISO 9				35 200 000	8 320 000	293 000

Figure 2.2 ISO Cleanroom Classifications.

2.2 Modes of Transmission of Airborne Diseases

A variety of airborne infections in susceptible hosts can result from exposures to clinically significant microorganisms released into the air when environmental reservoirs (i.e., soil, water, dust, and decaying organic matter) are disturbed. Once these materials are brought indoors into a healthcare facility by any of a number of vehicles (e.g.,people, air currents, water, construction materials, and equipment), the attendant microorganisms can proliferate in various indoor ecological niches and, if subsequently disbursed into the air, serve as a source for airborne healthcare-associated infections [1].

Respiratory infections can be acquired from exposure to pathogens contained either in droplets or droplet nuclei. Exposure to microorganisms in droplets (e.g., through aerosolized oral and nasal secretions from infected patients [29]) constitutes a form of direct contact transmission. When droplets are produced during a sneeze or cough, a cloud of infectious particles >5 μm in size is expelled, resulting in the potential exposure of susceptible persons within 3 feet of the source person [30]. Examples of pathogens spread in this manner are influenza virus, rhinoviruses, adenoviruses, and respiratory syncytial virus (RSV). Because these agents primarily are transmitted directly and because the droplets tend to fall out of the air quickly, measures to control air flow in a healthcare facility (e.g., use of negative pressure rooms) generally are not indicated for preventing the spread of diseases caused by these agents. Strategies to control the spread of these diseases are outlined in another guideline [31].

The spread of airborne infectious diseases via droplet nuclei is a form of indirect transmission [32]. Droplet nuclei are the residuals of droplets that, when suspended in air, subsequently dry and produce particles ranging in size from 1-5 μm. These particles can:

1. contain potentially viable microorganisms,

2. be protected by a coat of dry secretions,

3. remain suspended indefinitely in air,

4. be transported over long distances.

The microorganisms in droplet nuclei persist in favorable conditions (e.g., a dry, cool atmosphere with little or no direct exposure to sunlight or other sources of radiation). Pathogenic microorganisms that can be spread via droplet nuclei include Mycobacterium tuberculosis, VZV, measles virus (i.e., rubeola), and smallpox virus (i.e., variola major) [30]. Several environmental pathogens have life-cycle forms that are similar in size to droplet nuclei and may exhibit similar behavior in the air. The spores of Aspergillus fumigatus have a diameter of 2 μm-3.5 μm, with a settling velocity estimated at 0.03 cm/second (or about 1 meter/hour) in still air. With this enhanced buoyancy, the spores, which resist desiccation, can remain airborne indefinitely in air currents and travel far from their source [6].

3. AIR QUALITY MONITORING TECHNIQUES

3.1 Non-Viable Particle Monitoring Techniques

Non-viable particle monitoring can also be named with 'Particle Counting'. And the equipments used in non-viable particle monitoring are generally called 'Particle Counters'.

Cleanroom certification is an ongoing process. Continuously monitoring the air quality ensures the filtration system is working properly and that no unknown particle generators exist.

In the early days of clean manufacturing processes, test filters captured particles. Later, lab personnel used microscopes to confirm the number and size of the captured particles. Sometimes, the person counting the particles could determine the composition of the particles (e.g. copper dust). Negating the time-consuming efforts, microscopy is still the best way to learn specific information about particles, but does not offer instantaneous contamination data. Microscopy reveals historic, not current, particle events.

In the mid-1950s, military applications spawned the development of the first particle counting instruments. These devices made it possible to monitor instantaneous particle levels and provide quick notifications when contamination levels exceeded limits. Instead of waiting days for particle analysis, which could allow thousands of defective products to pass through a process, the particle counter provided data in minutes.

Gradually, this technology spread to other sectors of manufacturing and confidence grew in the new particle counter technology. Process engineers monitoring real-time particle contamination levels started to develop processes that were more

efficient, with less damaged product.

Today, the particle counter continuously improves productivity by providing detailed particle contamination levels, trends, and sources. Manufacturing personnel use particle data to understand causes of contamination, precisely schedule cleanroom maintenance cycles, correlate contamination levels with manufacturing processes, and fine-tune each step of production [2].

3.1.1 Types of Partical Counters

There are several varieties of particle counters. The primary differences depend upon the medium in which particles are suspended: air, liquid, gas, vacuum, or atmospheric / meteorological.

Airborne: Airborne particle counters measure contamination in HEPA-filtered cleanrooms, disk drive assemblies, drug manufacturers, small test benches, rocket launch facilities, and hundreds of different controlled air applications.

Liquid: Liquid particle counters measure contamination in everything including drinking water, injectable drugs, transmission fluids, and hydrofluoric acids. Some liquid particle counters require an accessory called a Sampler. A sampler communicates with the particle counter, automatically extracts a precise volume of liquid, and programmed with the counter's specific delivery rate, dispenses the liquid to the particle counter. Some liquid counters directly connect into plumbing lines or use pressurized gases to eliminate bubbles in chemicals.

Gas: Gas particle counters measure contamination suspended in gases. These gases may be either inert or volatile, and either dry (anhydrous) or contain trace water vapors. Usually, the gas particle counter's design provides contamination measurements at pressures ranging from 40-150 psig.

Vacuum: Vacuum particle counters fill a niche market where processes occur under negative pressures (vacuum), which offer unique challenges. Particles do not exhibit predictable movement in vacuum, so specialized particle counters must depend upon a particle's momentum for detection.

Atmospheric/Meteorological: One of the original particle counter applications, atmospheric or meteorological particle counters examine atmospheric contamination like pollution or provide detailed weather studies. These instruments measure water droplets, ice crystals, condensation nuclei, or contamination drift from oil fires and volcanic eruptions.

3.1.2 Comments Regarding Laser Particle Counters

Particle counters do not count particles. Particle counters count pulses of scattered light from particles, or in some cases, they count the shadows cast by backlit particles. The amount of light a particle scatters, or eclipses, can vary with several different factors, including the following:

1. The shape of the particle: Particles are seldom smooth and spherical like the PSL particles used in particle counter calibrations. Often, particles are flakes of skin or jagged fibers. When they float through the viewing volume sideways, they will scatter a different amount of light than if they travel through lengthwise.

2. The albedo (reflectivity) of the particle: Some particles are more reflective (e.g. aluminum) than others, which cause more scattered light onto the photodetector. The photodetector produces a larger pulse, and the particle counter thinks the particle is larger than its actual size. Conversely, some particles are less reflective (e.g. carbon) and the particle counter thinks a smaller particle passed through the viewing volume.

Particle counters do not count every particle within a volume. For instance, in a 5,000 ft^3 cleanroom with 12 foot ceilings, a 1.0 cubic-foot-per-minute (CFM) particle counter will analyze only 1/60,000 (or 0.0000167%) of the total room air in one minute. In an hour, the particle counter will count sixty times more air, which is equivalent to only 0.001% of the total room's volume. Considering only a small volume is sampled, particle counters should sample enough of the media (air, liquid, or gas) to statistically represent the entire volume. This is called statistical significance and is a valid representation of the entire volume [2].

3.1.3 Variations of Particle Counter Technologies

Several technological variations can be used when designing a particle counter. The application dictates the variant technology employed in the particle counter. In addition, the lasers chosen for variant technologies are selected for their particle sizing proficiency.

A laser's intensity is not uniform. Specifically, a laser is more intense at the center than at the edges. The laser's intensity illustrates a Gaussian or bell-shaped distribution. Some particle counters use special optical masks to view only the laser's center portion.

3.1.3.1 Scattering vs. Extinction. Both scattering and extinction technologies use a laser to illuminate a viewing area. Scattering particle counters measure a particle's reflected (scattered) light as it passes through the viewing region. Extinction particle counters illuminate the entire viewing volume and measure a particle's shadow (areas where light is extinct) as it passes through the viewing region. Extinction technology is only used in liquid particle counters that size particles larger than 2.0 μm. If scattering technology was used for large particles, the photodetector would be blinded by the intense scattered light.

3.1.3.2 Volumetric vs. Non-Volumetric. Volumetric particle counters examine the entire sample volume for particles. Non-volumetric particle counters look at only a small representative portion of the entire sample volume. Typically, non-volumetric particle counters have higher flow rates that allow more total volume to be sampled; conversely, they sacrifice some differentiation in particle size channels, which is called resolution. Volumetric particle counters usually sample liquid more slowly, but provide many particle sizing channels and better resolution.

3.1.3.3 Spectrometer vs. Monitor. Previously mentioned, the laser beam's intensity is not uniform throughout the beam's profile. Spectrometers use only the center of the laser beam, and monitors use the full width of the laser beam.

Spectrometers use the center portion of the laser beam because the laser's intensity is consistent. More consistent light sources provide greater accuracy in particle detection, so a spectrometer may easily discern slight differences in particle sizes and offer better resolution.

Monitors use the entire laser beam, so they cannot perceive small differences between particle sizes. Illustrated in Figure 3.1, a particle passing through the laser beam's edge will be subject to lower-intensity light than the same particle passing through laser beam's center. The relative pulse amplitudes, shown below the diagram, illustrate a particle's pulse and the noise floor (background electrical noise). As shown, the same particle will create different pulse amplitudes depending upon where it enters the laser beam. Similarly, a larger particle passing at the beam's edge may provide the same pulse amplitude as a small particle passing through the beam's center. Consequently, monitors include only a few sizing channels, with enough distance between channels, to account for this sizing error.

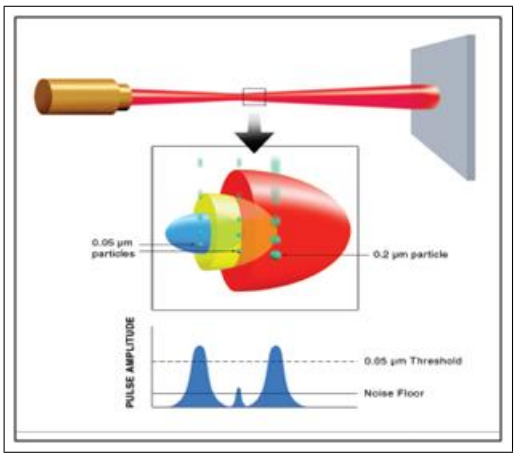

Figure 3.1 Laser Intensity and Sizing Errors.[2]

3.2 Viable Particle Monitoring Techniques

The assay of the microbial content of the air has become increasingly more significant in the past decade as the need for 'contamination-free' environments has become more apparent. The treatment of hospital patients, medical as well as surgical, who are high risk candidates for infection; the manufacture and processing of sterile materials and pharmaceuticals, and the increased use of these products; the massive production and wide distribution of convenience foods; and the growing emphasis on consumer protection have all contributed to the need for controlled environments. Biological aerosols have been defined as viable biological contaminants occurring as solid or liquid particles in the air. These particles can vary in size from viruses less than 0.1 micron in diameter to fungal spores 100 or more microns in diameter. They may occur as single, unattached organisms or as aggregates.

Viable particle samplers have been generally used to collect and assay aerobic species of bacteria and fungi. Even though many viable samplers will collect some virus particles, there is no convenient, practical method for the cultivation and enumeration of these particles.

There are two constraints on all viable particle samplers for which there is no analog in the assay of non-biological aerosols. First, the particle must be separated

from the air for any viability assay, and second, the ability to reproduce (viability) must be demonstrated.

There are a great variety of methods available for the collection and enumeration of airborne viable particles [33]. The selection of a particular method and device will depend upon the purpose for which the sample is required. The collection efficiency of samplers will vary; an appropriate method or methods and equipment should be carefully selected. Sampling devices fall into two categories:

1. passive sampling devices, such as settle plates,
2. active sampling devices, such as impact, impingement and filtration samplers.

3.2.1 Passive microbial sampling devices

Passive microbial air sampling devices such as settle plates do not measure the total number of viable particles in the air; they measure the rate at which viable particles settle on surfaces. Settle plates may therefore be used for the qualitative and quantitative evaluation of airborne contamination of products. This can be done by determining the settle plate count per time; then, by relating both the area and time of exposure of the product to that of the settle plate, the possible contamination of the product can be calculated [34]-[35] (Figure 3.3).

3.2.2 Active microbial sampling devices

3.2.2.1 General. The use of active air sampling devices in risk zones is essential for the assessment of the microbial quality of air. There are several types of active devices commercially available, each having its own limitations.

Based on the principles of sampling, the two main types of apparatus considered

Figure 3.2 An example of an incubated settle plate.

suitable for risk zones with normal (low level) biocontamination are impact samplers and filtration samplers [36].

3.2.2.2 Impact and impingement samplers. Because there are a variety of impact and impingement samplers available for the detection of viable particles, the device selected for use should have the following characteristics:

1. impact velocity of the air hitting the culture medium that is a compromise between
   (a) being high enough to allow the entrapment of viable particles down to approximately 1 μm,
   (b) being low enough to ensure viability of viable particles by avoiding mechanical damage or the breakup of clumps of bacteria or micromycetes.
2. sampling volume that is a compromise between being large enough to detect very low levels of bio contamination and being small enough to avoid physical or chemical degradation of the collection medium.

In areas of high biocontamination, the impaction method and sample volume should be selected in way appropriate to achieving separate colonies, to allow the results

to be interpreted.

The device should meet the following minimum requirements:

1. sufficient flow rate to collect 1 m^3 in a reasonable time, without significant drying of the sampling medium,
2. appropriate air impact speed to the culture medium [36].

Figure 3.3 Single-Stage Impact Sampler.[3]

3.2.2.3 Filtration samplers. Filtration sampling devices are widely used for air sampling. By appropriate choice of pump, filter medium and filter size, almost any desired sample quantity can be collected in a given sampling period.

For the design and use of a filtration sampling device, the following factors should be considered:

1. The filtration conditions do not affect the viability of the microorganisms collected, e.g. by dehydration;

2. Static electricity that will interfere with the rate of impact of viable particles onto the filter membrane must be eliminated;

3. The same constraints or suction flow rate and impact air velocity must be applied

4. The filter membrane holder must be connected to a vacuum source, fitted with a device for measuring the suction rate, without contamination of the filter material;

5. The filter membranes must be placed aseptically in the filter holder and removed aseptically after filtering the desired quantity of air and must be placed on solid culture medium or in liquid culture medium [36].

4. METHODOLOGY

4.1 Environment

Both particle counting and microbiological sampling measurements are performed simultaneously in five operating rooms, in three hospitals. Three of the rooms rank ISO Class 6 (Hospital-1 Room-1, Hospital-2 Room-1 and Hospital-2 Room-2) and two of the rooms rank ISO Class 7 (Hospital-3 Room-1 and Hospital-3 Room-2). In each operating room; four measurements are taken before the operation (Figure 4.1), and four measurements are taken during surgery (Figure 4.3). In each of the operations, six personnel performed the operation.

Figure 4.1 'At-Rest' measurement.

Four measurements are taken from each corner of the operating table, where most particle and microbiological aerosols are likely to be detected [14] (Figure 4.2).

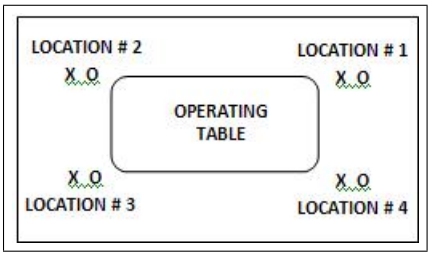

Figure 4.2 Measurement sites in the operating room. 'X' represents position of the particle counter and 'O' represents position of the cascade impactor.

ISO 14644-1 states that in a A (m^2) base area operating room, a minimum of $\sqrt{A}$ measurements should be taken for particle counting [28]. As our measurement site near the operating table is considered, the area across the operating table does not exceed 16 m^2 in all of the five operating rooms.

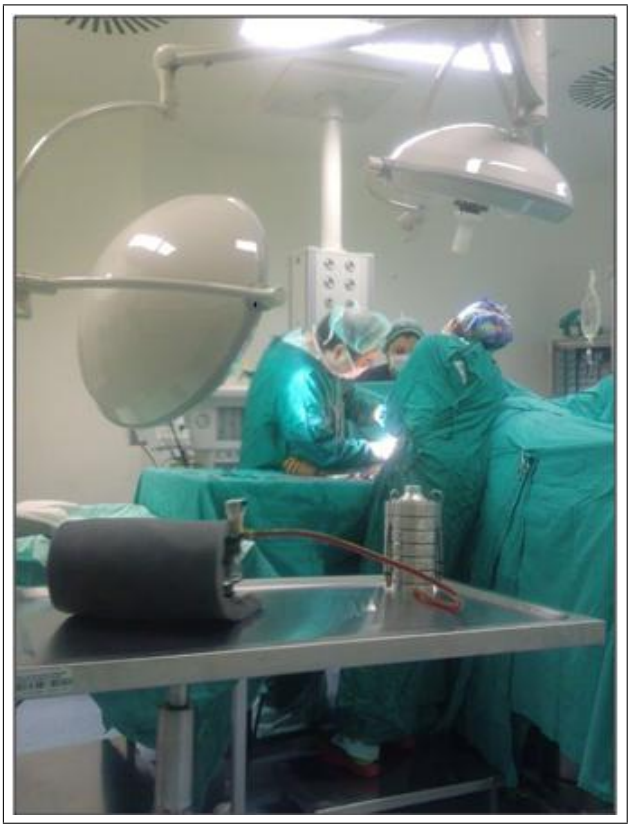

Figure 4.3 Measurement during surgery.

4.2 Apparatus

4.2.1 Particle Counter

For particle counting, Particle Measurement Systems, model LASAIR III 350 L particle counter is used (Figure 4.4). This counter has a flow rate of 50.0 L / min. The particle counter has 6 channels which can classify the particles into 6 groups according to the size of particles: $\geq 0.3\mu$m, $\geq 0.5\mu$m, $\geq 1.0\mu$m, $\geq 5.0\mu$m, $\geq 10.0\mu$m and $\geq 25.0\mu$m.

Figure 4.4 LASAIR III Particle Counter.

4.2.1.1 Particle Counting Methodology. Measurements are repeated three times at each location (Four locations in five operating rooms during at rest and operational measurement). Totally, a number of 120 measurements are taken. The particle counter is placed at a height of 1 meter above the floor and the sampling duration of each of the 120 measurements are one minute, so 50 liters of air is sampled. The particle counter prints out a detailed report including particle counts at each of the channel. In order to have same measurement quantities with the microbiological sampling measurements, the average of three measurements at the same location is used in data analysis.

4.2.2 Viable Particle Sampler

The New-Star Andersen Six-stage Viable Particle Sampler is a multi-orifice, cascade impactor which is normally used to measure the concentration and particle size distribution of aerobic bacteria and fungi in the intramural or ambient air (Figure 4.5). This instrument has been widely used as a standard for enumerating the viable particles in a microbial aerosol. Viable particles can be collected on a variety of bacteriological agar and incubated in situ for counting and identification. This sampler is calibrated so that all particles collected, regardless of physical size, shape, or density are sized aerodynamically and can be directly related to human lung deposition.

Figure 4.5 New-Star Andersen Six-Stage Viable Counter used in the experiments.

The human respiratory tract is an aerodynamic classifying system for airborne particles [37]. A sampling device can be used as a substitute for the respiratory tract as a collector of viable airborne particles and as such, it should reproduce to a reasonable degree the lung penetration by these particles. The fraction of inhaled particles, retained in the respiratory system and the site of deposition vary with all the physical properties (size, shape, density) of the particles which make up the aerodynamic dimensions [37].

Because the lung penetrability of unit density particles is known [38] and since

the particle sizes that are collected on each stage of the Viable Samplers have been determined, if a standard model of these samplers is used according to standard operating procedure, the stage distribution of the collected material will indicate the extent to which the sample would have penetrated the respiratory system.

Numerous small round jets improve collection (impaction) efficiency and provide a sharper cutoff of particle sizes on each stage of inertial impactors [39]. Thus, the Six-Stage Sampler with 400 small round jets per stage meets all the criteria for the efficient collection of airborne viable particles. Reports have discussed a reduced efficiency in cascade impactors when particles bounce off the impaction surface, are re-entrained and lost in the exhaust air [40]. This effect is minimized when a sticky agar surface is used as the collection medium.

The jet orifice diameters and the particle sizes are tabulated in Table 4.1 [3].

Table 4.1
Jet Orifice Diameters and Range of Particle Sizes of Six-Stage Viable Sampler.

Stage	Orifice Diameter (mm)	Range of Particle Sizes(μm)
1	1.81	≥ 7.1
2	0.91	4.7-7.1
3	9.71	3.3-4.7
4	0.53	2.1-3.3
5	0.34	1.1-2.1
6	0.25	0.65-1.1

4.2.2.1 Microbiological Sampling Methodology.

1. Petri dishes are sterilized prior to filling. Also, all six stages of the cascade impactor are cleaned by using soap or detergent.

2. Collection plates are filled with 27 ml of sterile bacteriological agar.

3. Sabouraud's Dextrose Agar (SDA) is used for stages 1 and 2 to detect fungal population. The reason is, the first two stages catch the particles larger than 4.7 μm and most of fungal organisms have a size larger than 5.0 μm [41].

4. Sheep Blood Agar %5 is used for stages 3,4,5 and 6 to detect bacteria.

5. Each plate is inserted at the corresponding stage of the cascade impactor.

6. The vacuum pump is calibrated to supply a constant amount of air 1 ACFM (28.3 $\pm$ 5% liters/min) .

7. The sampling period is 20-25 minutes for each location. Extended sampling periods are not used, because this would correspond to dehydrate the plates and damage the viable particles that are already collected.

8. After sampling, each petri dish is identified and numbered (Figure 4.6)

Figure 4.6 Petri dishes after exposure for 25 minutes.

9. The agar plates are then incubated at 37 °C for 72 hours for Sabouraud's Agar; and at 37 °C for 24 hours for Blood Agar.

10. Following incubaion, the mean number of viable particles is counted for each particle size. [3].

11. This procedure is applied to four locations, in five operating rooms, under 'at-rest' and operational conditions.

5. RESULTS

Particle counting and microbiological sampling methods are performed simultaneously in rooms A,B,C,D and E 'at-rest' and during surgery. AP and AO refers to measurements in room A, 'at-rest' and during surgery respectively. 60 particle counting measurements and 120 microbiological samplings are realized. Results are shown below.

Table 5.1
'At-rest' Particle Counting Results. Results are cumulative for each size. (* 12 measurements at each location)

Concentrations (Particles / m^3)						
Locations	$\geq 0.3\mu$m	$\geq 0.5\mu$m	$\geq 1\mu$m	$\geq 5\mu$m	$\geq 10\mu$m	$\geq 25\mu$m
AP-Mean*	137049	21694	8274	1254	519	39
BP-Mean*	48941	9665	4020	725	385	35
CP-Mean*	95586	18475	6740	950	425	40
DP-Mean*	584140	55985	16566	1330	325	25
EP-Mean*	567982	54829	16523	1393	250	25
Mean	**286739.3**	**32129.2**	**10424.45**	**1130.2**	**380.7**	**32.7**

Table 5.2
'During Surgery' Particle Counting Results. Results are cumulative for each size.(* 12 measurements at each location)

Concentrations (Particles / m^3)						
Locations	$\geq 0.3\mu$m	$\geq 0.5\mu$m	$\geq 1\mu$m	$\geq 5\mu$m	$\geq 10\mu$m	$\geq 25\mu$m
AO-Mean*	5958393	1211593	476812	62142	19438	4885
BO-Mean*	3611685	661785	196412	16475	7869	1060
CO-Mean*	790445	405940	195456	22840	8880	975
DO-Mean*	1458957	360573	162707	23980	7645	730
EO-Mean*	1639827	450266	204267	22681	8658	1175
Mean	**2691861**	**618031**	**247131**	**29623**	**10498**	**1765**

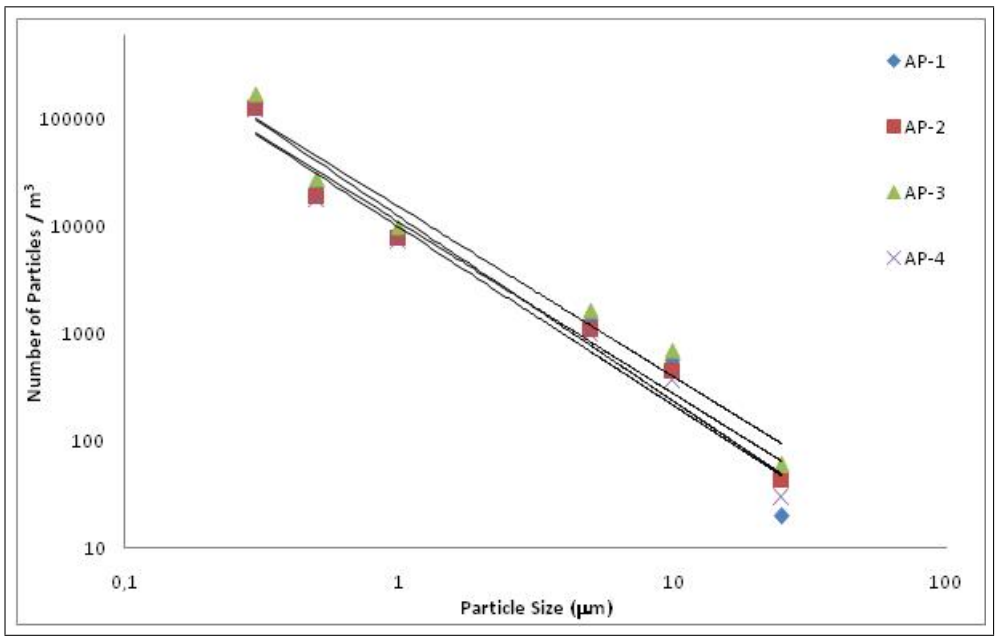

Figure 5.1 'At-Rest' particle counting results in Room A.

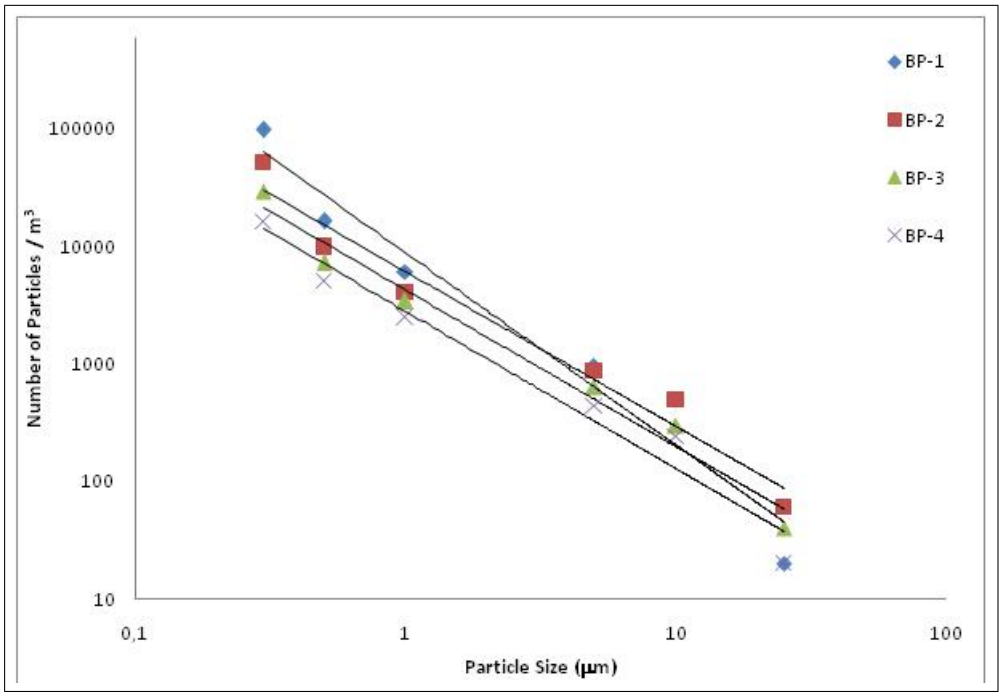

Figure 5.2 'At-Rest' particle counting results in Room B.

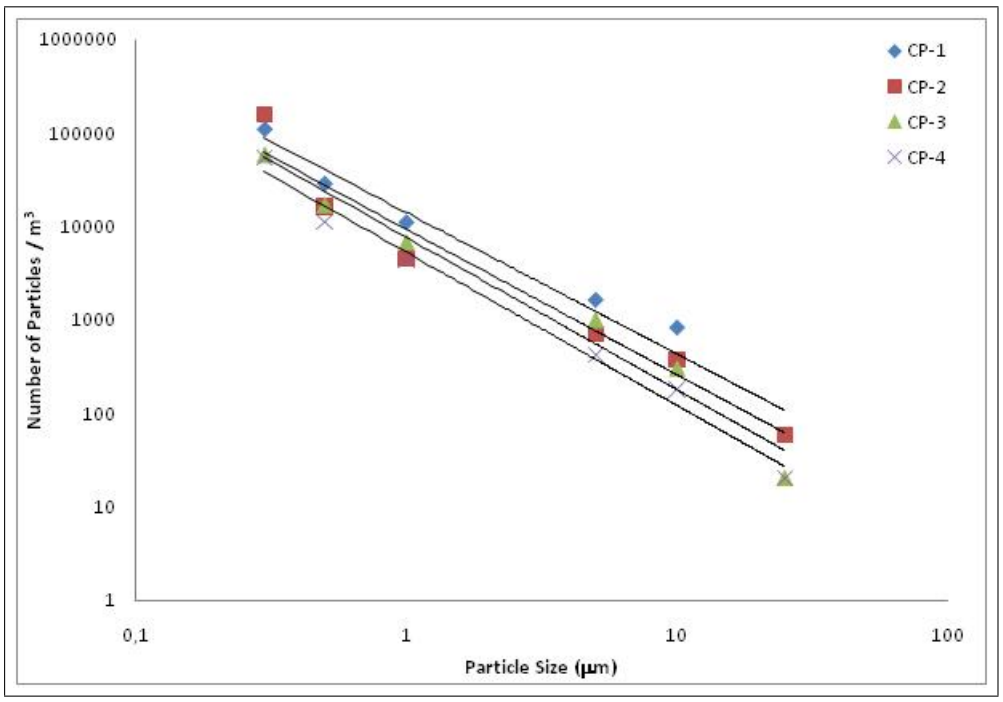

Figure 5.3 'At-Rest' particle counting results in Room C.

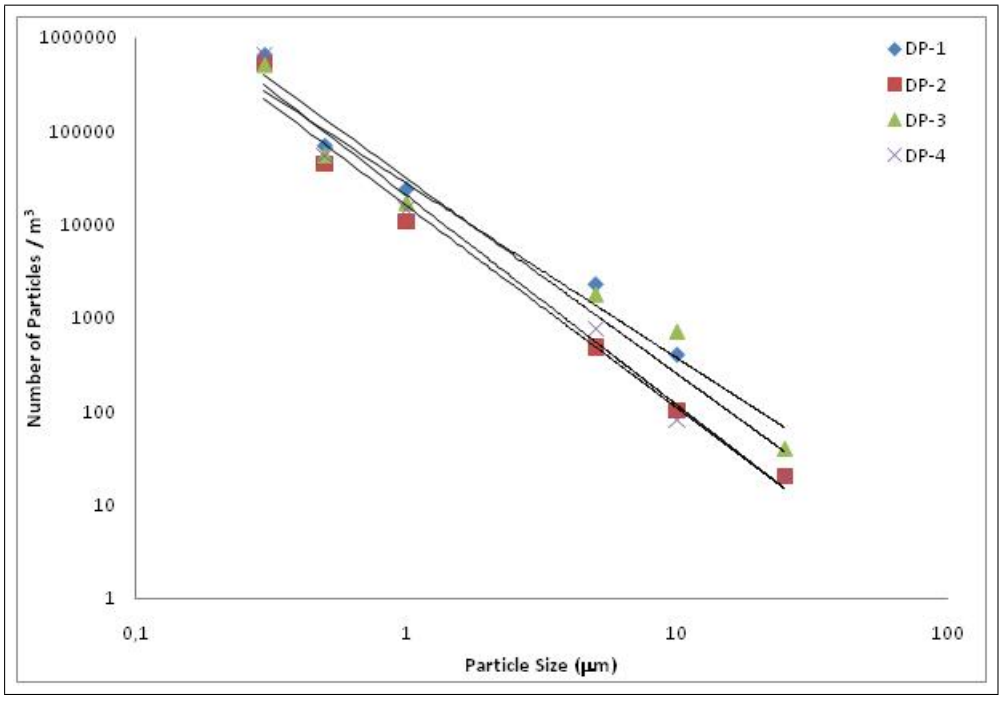

Figure 5.4 'At-Rest' particle counting results in Room D.

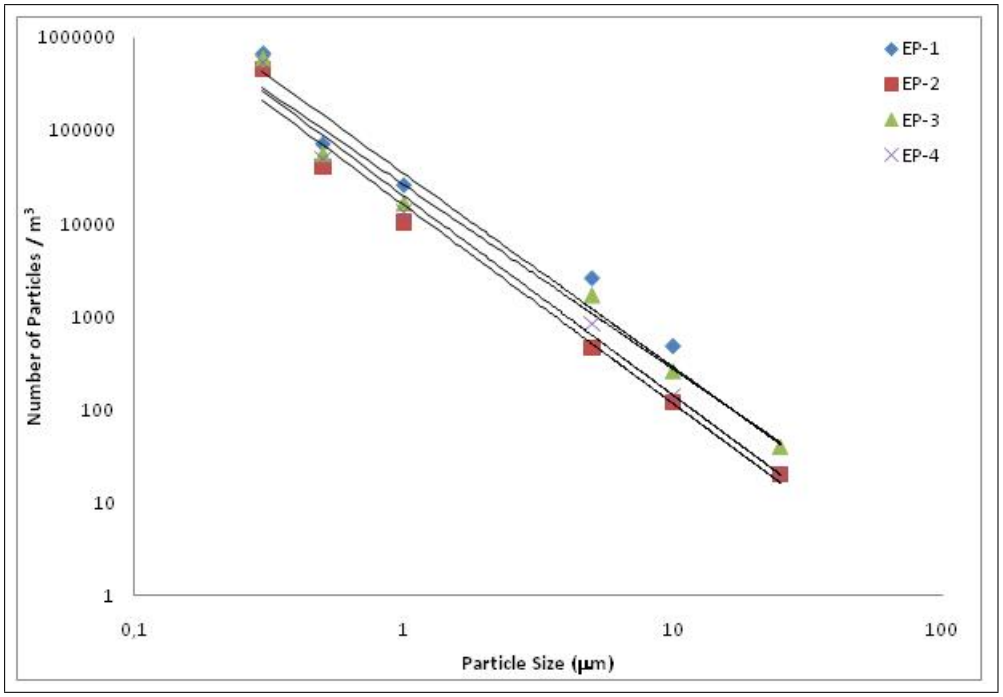

Figure 5.5 'At-Rest' particle counting results in Room E.

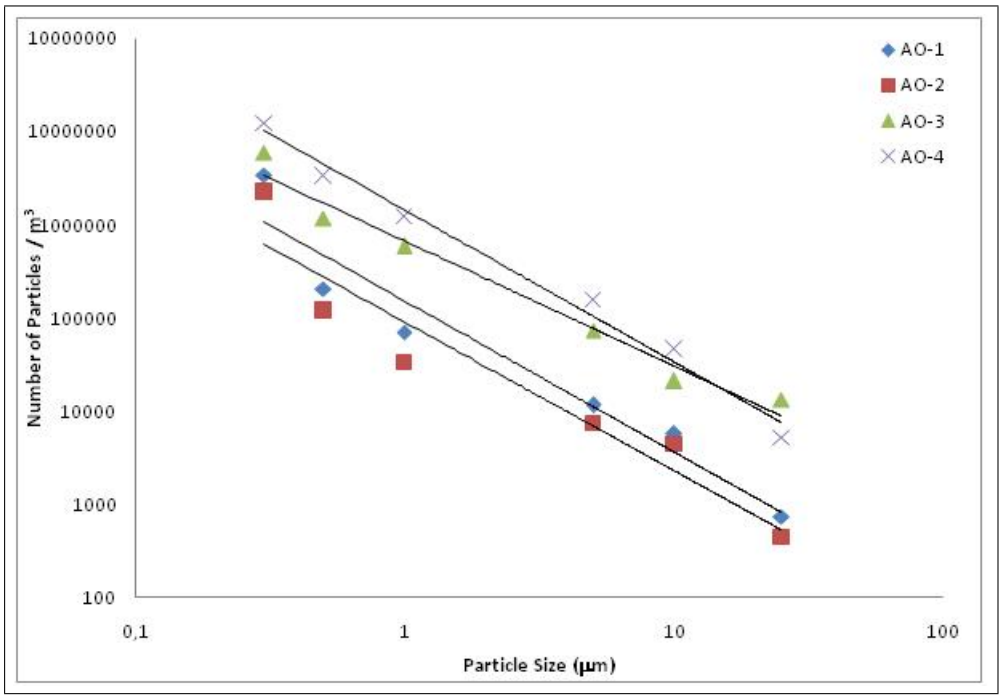

Figure 5.6 'During Surgery' particle counting results in Room A.

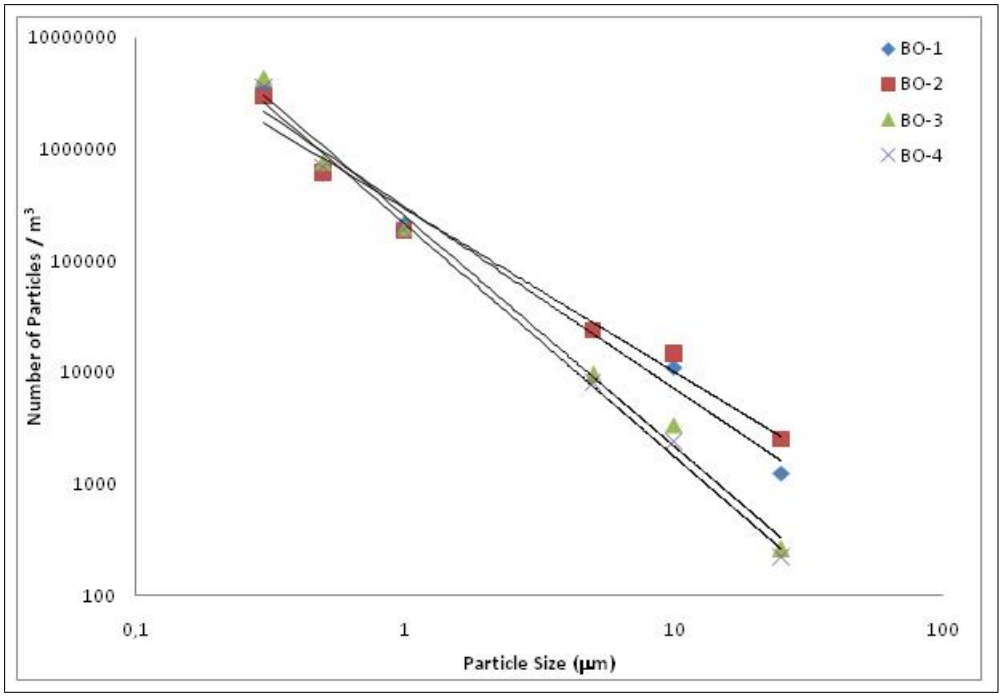

Figure 5.7 'During Surgery' particle counting results in Room B.

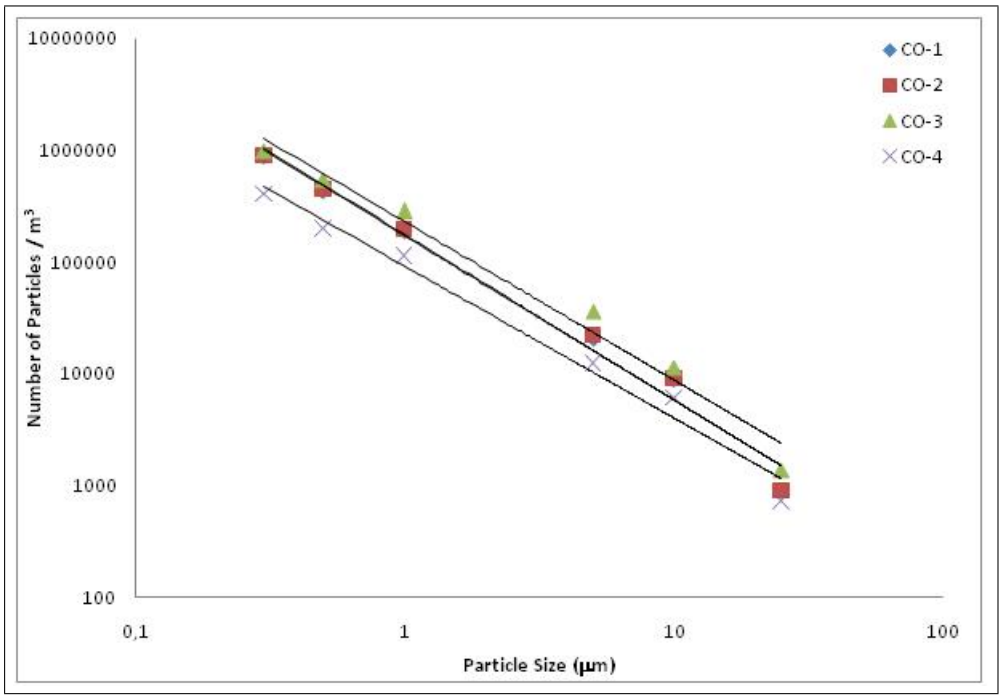

Figure 5.8 'During Surgery' particle counting results in Room C.

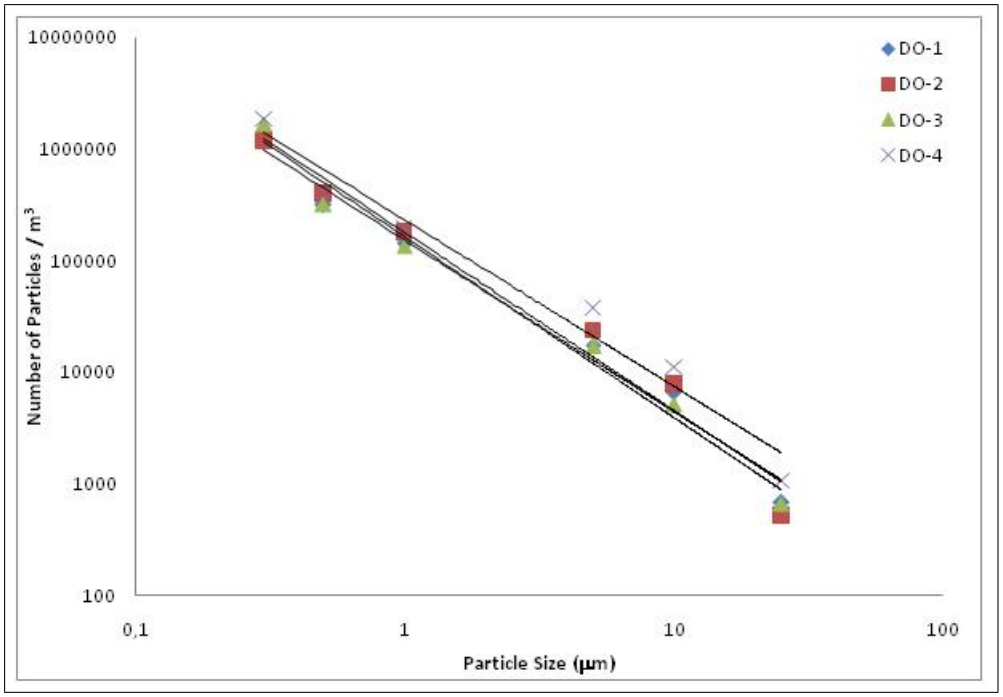

Figure 5.9 'During Surgery' particle counting results in Room D.

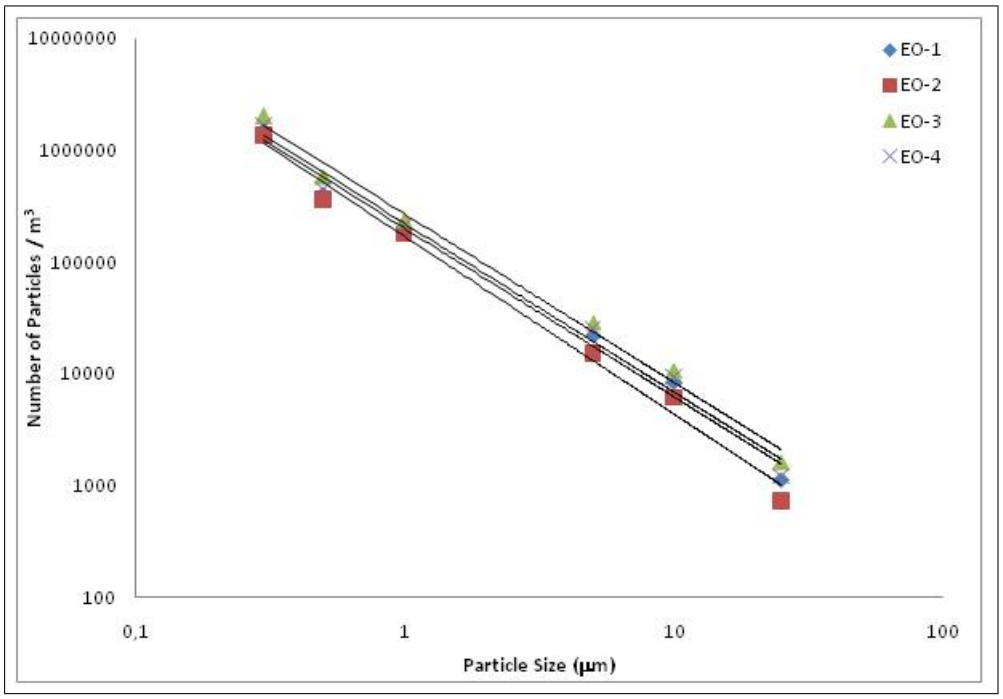

Figure 5.10 'During Surgery' particle counting results in Room E.

Table 5.3
'At-Rest' Microbiological Sampling Results. Results are cumulative for each particle size. (* 24 petri dishes are used at each location)

Mean CFU / m^3)						
Locations	$\geq 0.65\mu$m	$\geq 1.1\mu$m	$\geq 2.1\mu$m	$\geq 3.3\mu$m	$\geq 4.7\mu$m	$\geq 7.1\mu$m
AP-Mean*	0.053	0.047	0.035	0.021	0.000	0.000
BP-Mean*	0.048	0.042	0.026	0.013	0.000	0.000
CP-Mean*	0.068	0.060	0.034	0.015	0.000	0.000
DP-Mean*	0.035	0.031	0.019	0.008	0.000	0.000
EP-Mean*	0.055	0.050	0.032	0.015	0.000	0.000
Mean	**0.052**	**0.046**	**0.029**	**0.014**	**0.000**	**0.000**

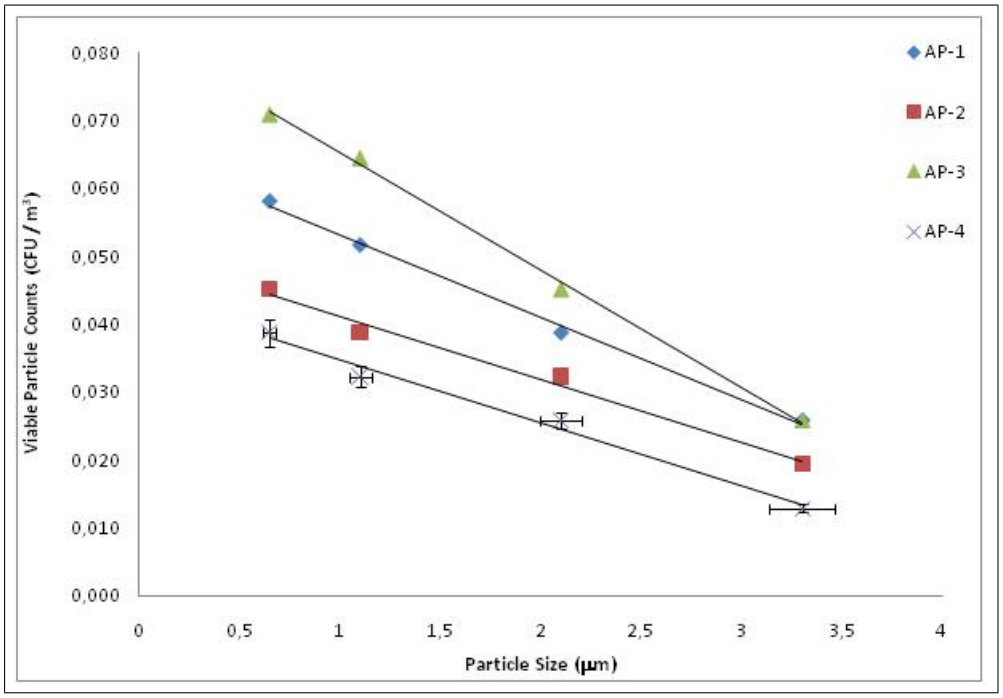

Figure 5.11 'At-Rest' microbiological sampling results in Room A.

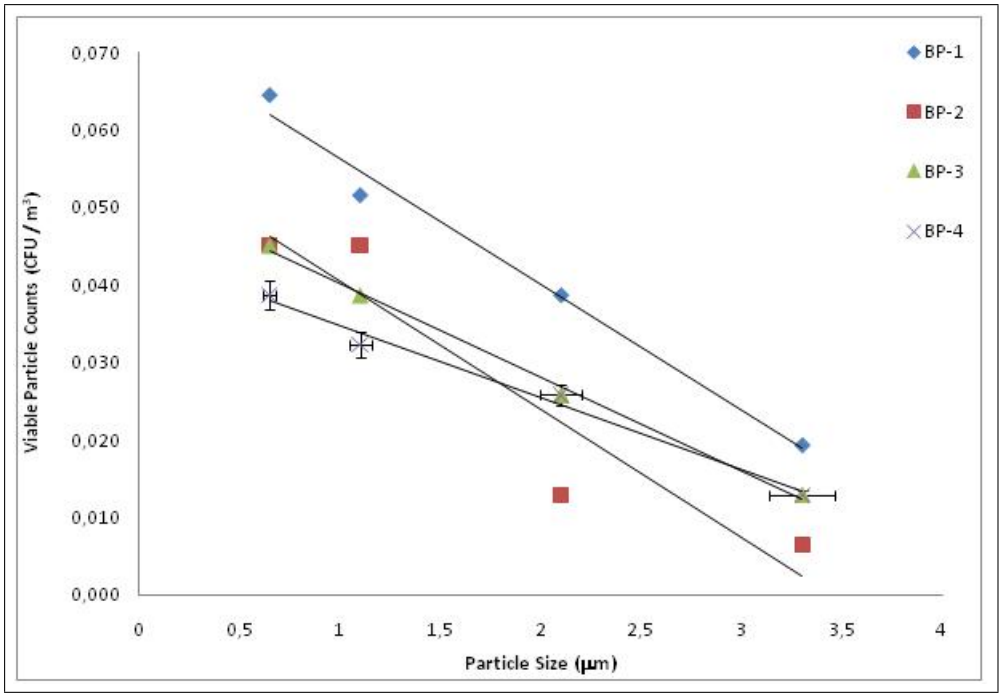

Figure 5.12 'At-Rest' microbiological sampling results in Room B.

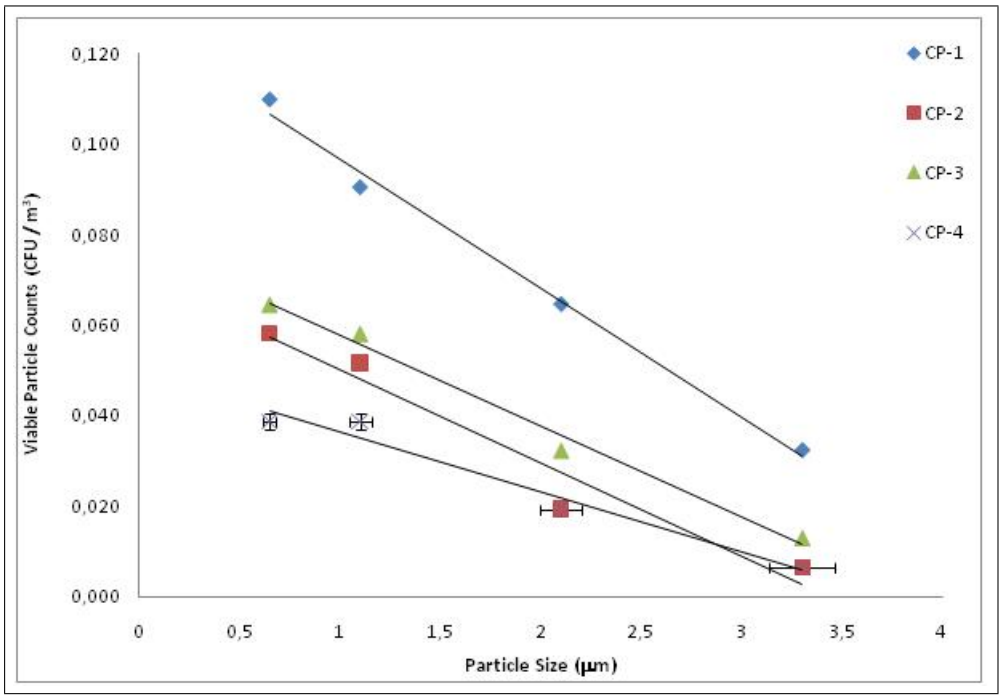

Figure 5.13 'At-Rest' microbiological sampling results in Room C.

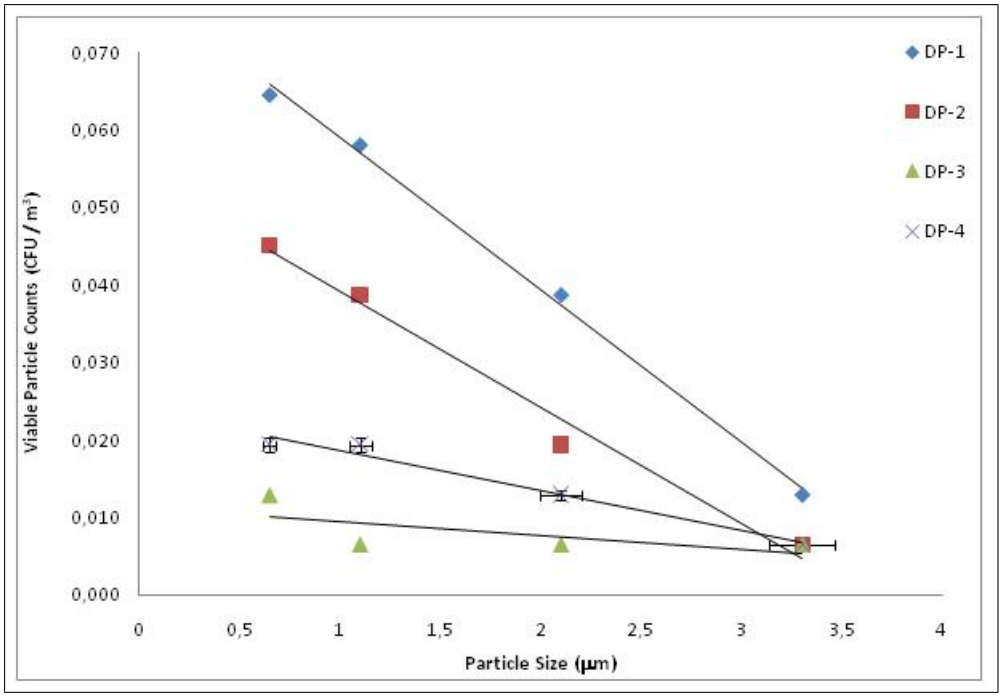

Figure 5.14 'At-Rest' microbiological sampling results in Room D.

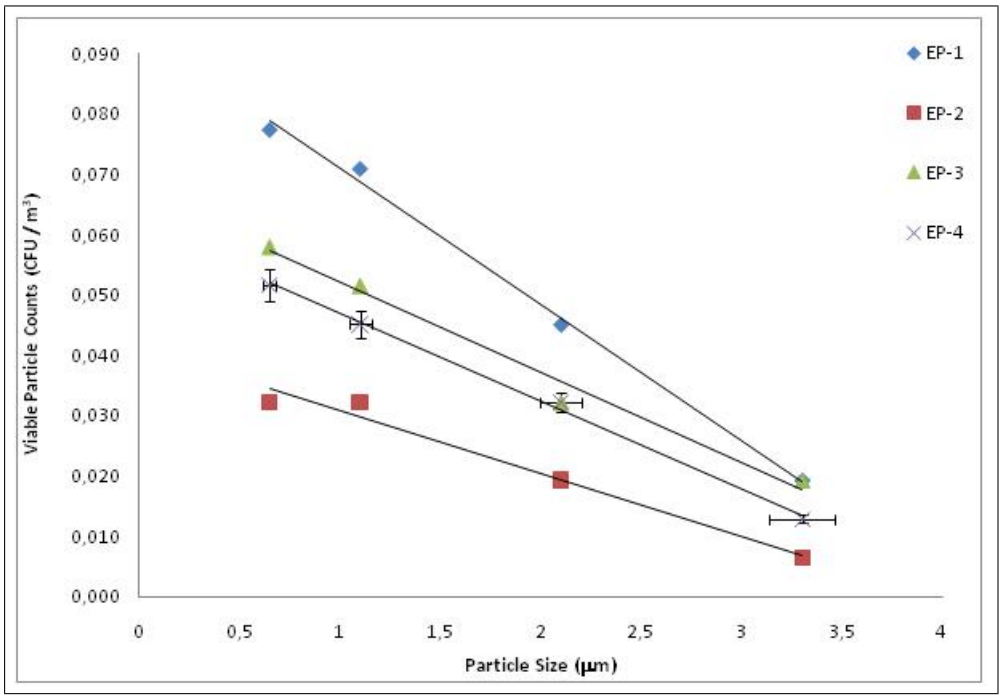

Figure 5.15 'At-Rest' microbiological sampling results in Room E.

Table 5.4
'During Surgery' Microbiological Sampling Results. Results are cumulative for each particle size. (* 24 petri dishes are used at each location)

Mean CFU / m^3)						
Locations	$\geq 0.65\mu$m	$\geq 1.1\mu$m	$\geq 2.1\mu$m	$\geq 3.3\mu$m	$\geq 4.7\mu$m	$\geq 7.1\mu$m
AO-Mean*	0.302	0.297	0.234	0.137	0.000	0.000
BO-Mean*	0.405	0.376	0.290	0.140	0.000	0.000
CO-Mean*	0.576	0.550	0.403	0.173	0.000	0.000
DO-Mean*	0.208	0.187	0.055	0.016	0.000	0.000
EO-Mean*	0.315	0.289	0.127	0.053	0.000	0.000
Mean	**0.361**	**0.340**	**0.222**	**0.140**	**0.000**	**0.000**

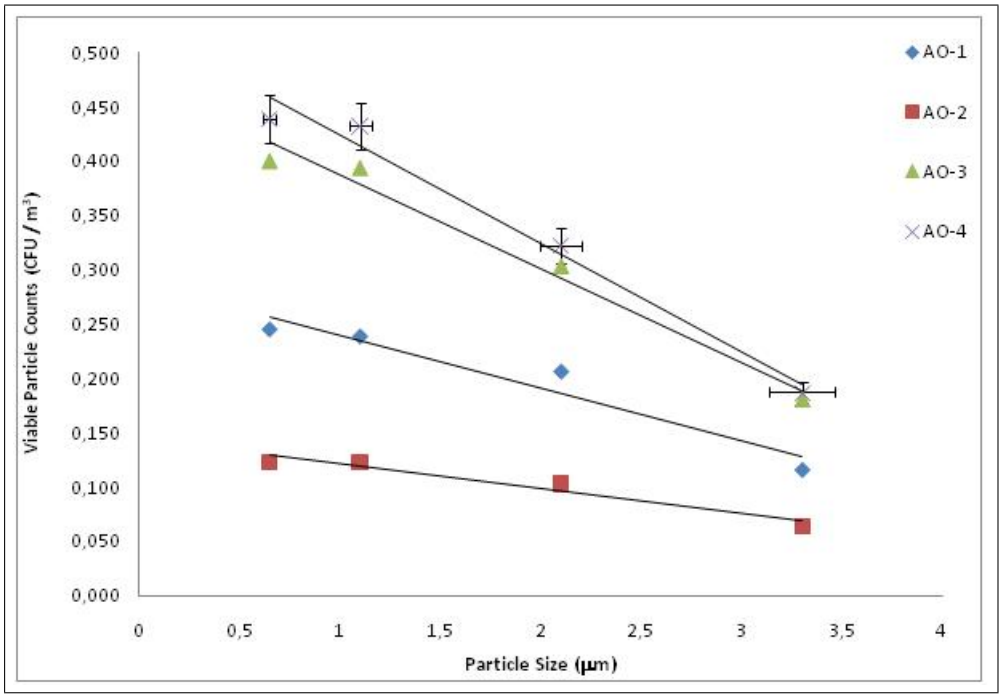

Figure 5.16 'During Surgery' microbiological sampling results in Room A.

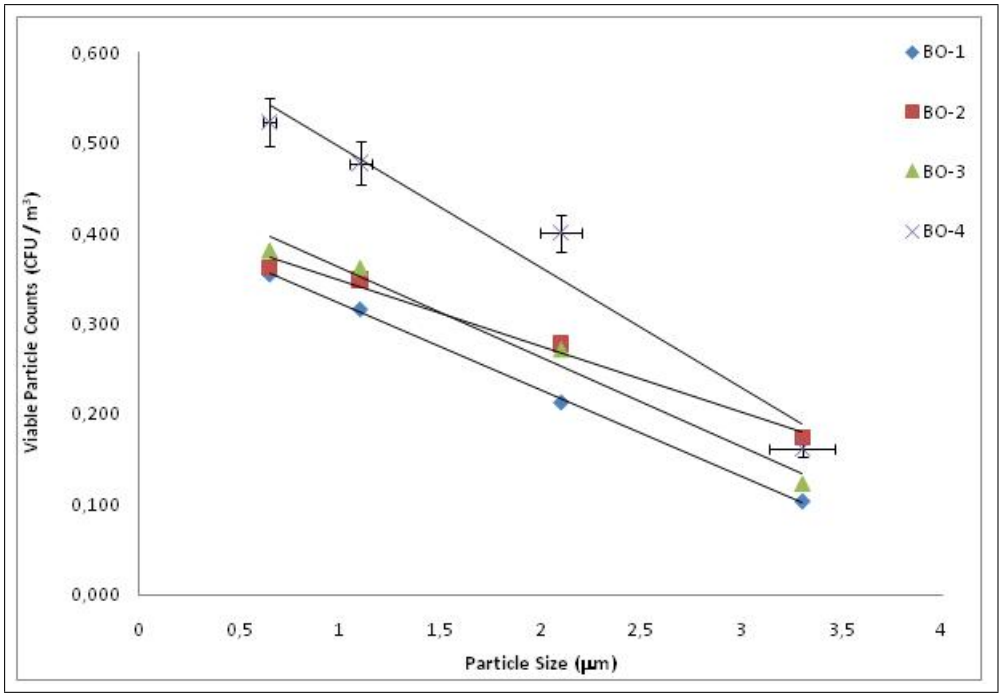

Figure 5.17 'During Surgery' microbiological sampling results in Room B.

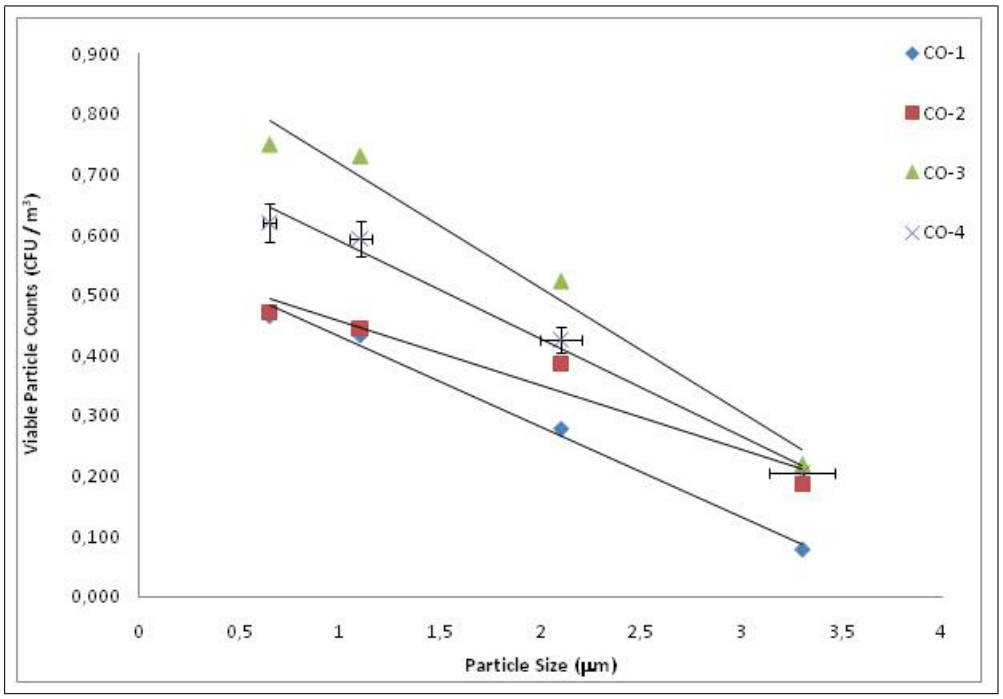

Figure 5.18 'During Surgery' microbiological sampling results in Room C.

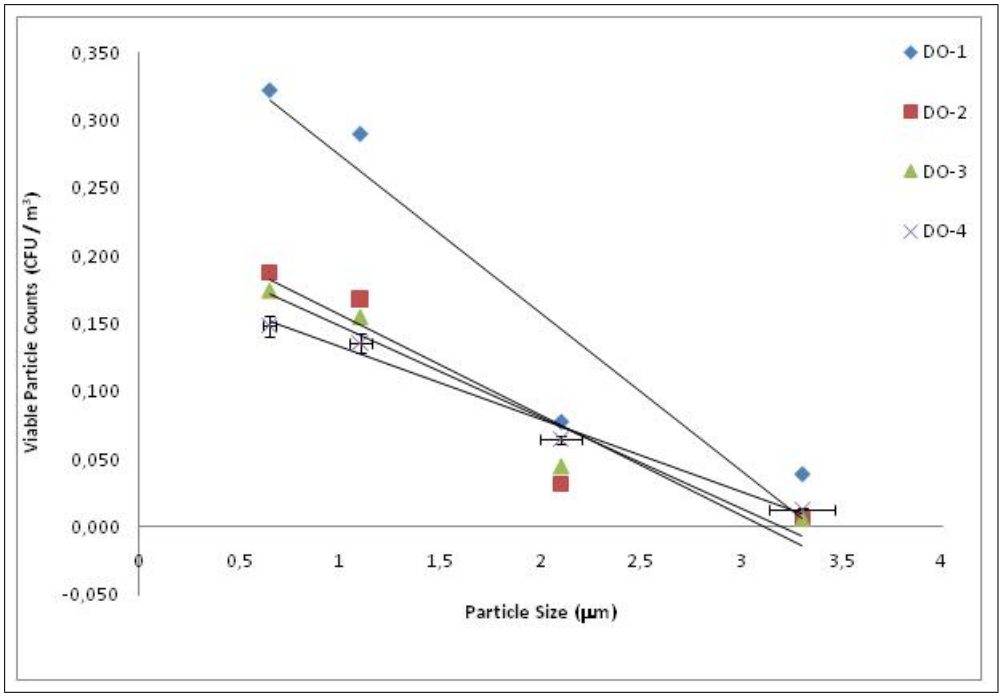

Figure 5.19 'During Surgery' microbiological sampling results in Room D.

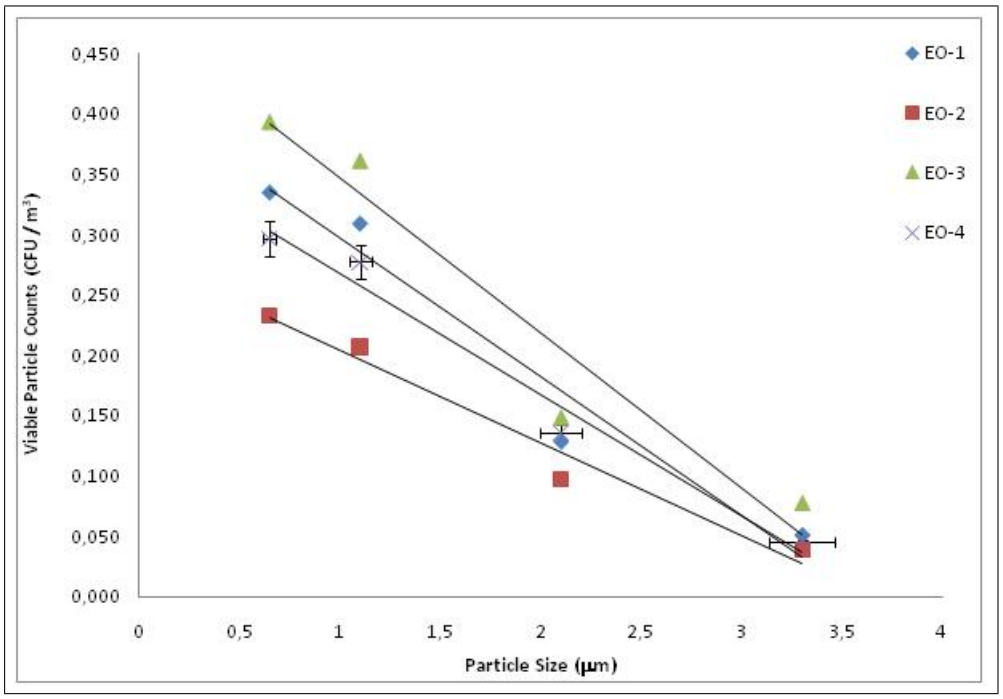

Figure 5.20 'During Surgery' microbiological sampling results in Room E.

6. DISCUSSION

To compare the two measurement techniques, since particle counter and microbiological sampler have different size range channels, a mathematical manipulation is required (Table 6.1).

Table 6.1
Comparison of the size ranges of the Particle Counter and Microbiological Sampler.

Stage	Particle Counter Channel Size Range (μm)	Viable Sampler Channel Size Range (μm)
1	$\geq$ 25.0	$\geq$ 7.1
2	10.0-25.0	4.7-7.1
3	5.0-10.0	3.3-4.7
4	1.0-5.0	2.1-3.3
5	0.5-1.0	1.1-2.1
6	0.3-0.5	0.65-1.1

To remedy this problem, log particle concentrations vs. log particle size graph is constructed (Figure 6.1)[42]. It is clearly seen from Figure 6.1 that the log-log relationship is perfectly linear, with a coefficient of regression R^2=0.97.

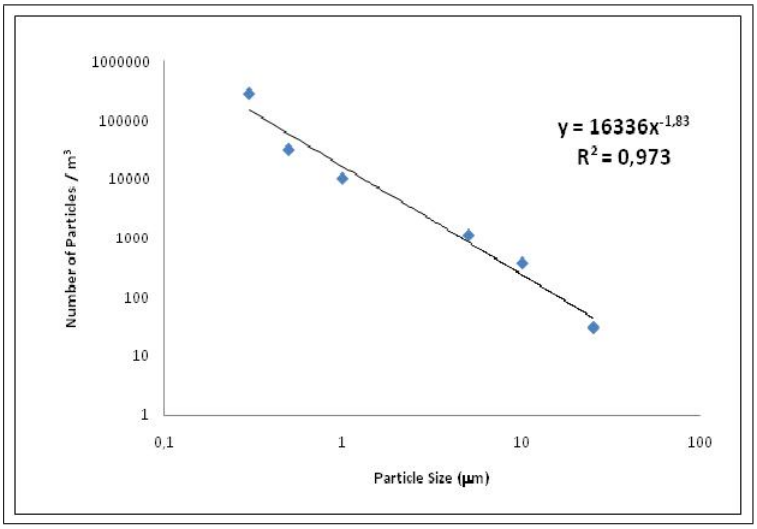

Figure 6.1 'At-Rest'particle counting measurement(AP-1).

Data from 40 particle counting measurements are plotted similarly to Figure 6.1 and an empirical equation of the form $y=Ax^b$ describes the relationship between number of particles and particle diameters (Table 6.2). By making use of this equation, the particle counting results are easily transformed into the same particle size ranges as the microbiological sampler.(Table 6.3 and Table 6.4).

Table 6.2
The coefficients of the equation $y=Ax^b$ derived for the particle counter measurements.

At-Rest			During Surgery		
Locations	A	b	Locations	A	b
AP-1	12160	-1.72	AO-1	149994	-1.62
AP-2	10737	-1.59	AO-2	89920	-1.60
AP-3	15022	-1.57	AO-3	681762	-1.35
AP-4	9838	-1.65	AO-4	1445242	-1.62
BP-1	8939	-1.65	BO-1	302226	-1.62
BP-2	6203	-1.33	BO-2	291695	-1.46
BP-3	4259	-1.33	BO-3	252022	-2.06
BP-4	2794	-1.34	BO-4	217623	-2.08
CP-1	14255	-1.51	CO-1	172555	-1.48
CP-2	9382	-1.56	CO-2	178413	-1.48
CP-3	7741	-1.63	CO-3	230642	-1.42
CP-4	5304	-1.64	CO-4	91852	-1.36
DP-1	32481	-2.10	DO-1	153683	-1.54
DP-2	16413	-2.17	DO-2	179176	-1.59
DP-3	27914	-1.88	DO-3	164933	-1.62
DP-4	20806	-2.25	DO-4	232541	-1.49
EP-1	34856	-2.09	EO-1	203118	-1.51
EP-2	15899	-2.14	EO-2	169692	-1.59
EP-3	26217	-1.99	EO-3	268114	-1.51
EP-4	19782	-2.15	EO-4	221769	-1.51

By using Table 5.3, Table 5.4, Table 6.3 and Table 6.4; viable microorganism counts are plotted against particle counts in 'At-Rest' (Figure 6.2, Figure 6.3, Figure 6.4, Figure 6.5) and 'During Surgery' conditions (Figure 6.6, Figure 6.7, Figure 6.8, Figure 6.9). Viable particles of the size 4.7 μm - 7.1 μm and 7.1 μm are not detected.

Table 6.3
'At-Rest' particle counting results normalized to the same range as Microbiological Sampling Sizes. The values are not cumulative and represent the number of particles between given size range.

Particle Counts(N/m^3) - At-Rest						
Locations	0.65-1.1(μm)	1.1-2.1(μm)	2.1-3.3(μm)	3.3-4.7(μm)	4.7-7.1(μm)	$\geq$ 7.1(μm)
AP-Mean*	13868	6654	1857	748	471	493
BP-Mean*	5590	2936	904	392	265	333
CP-Mean*	10148	5034	1453	600	385	425
DP-Mean*	39844	14772	3179	1067	570	431
EP-Mean*	39505	14670	3147	1050	557	411

Table 6.4
'During Surgery' particle counting results normalized to the same range as Microbiological Sampling Sizes. The values are not cumulative and represent the number of particles between given size range.

Particle Counts(N/m^3) - At-Rest						
Locations	0.65-1.1(μm)	1.1-2.1(μm)	2.1-3.3(μm)	3.3-4.7(μm)	4.7-7.1(μm)	$\geq$ 7.1(μm)
AO-Mean*	641906	321833	94271	39402	25684	29571
BO-Mean*	350980	151990	39048	15031	9161	9309
CO-Mean*	166590	88948	27652	12027	8112	10014
DO-Mean*	199589	99827	29057	12057	7790	8687
EO-Mean*	3229675	116929	34637	14550	9506	10865

For statistical analysis, data are tested for normal distribution. Lillie Test is applied to the data tabulated in Table 5.1, Table 5.2, Table 5.3 and Table 5.4; p and h values are given in Table 6.5.

Lillie Test showed us that the data retained in this study statistically do not

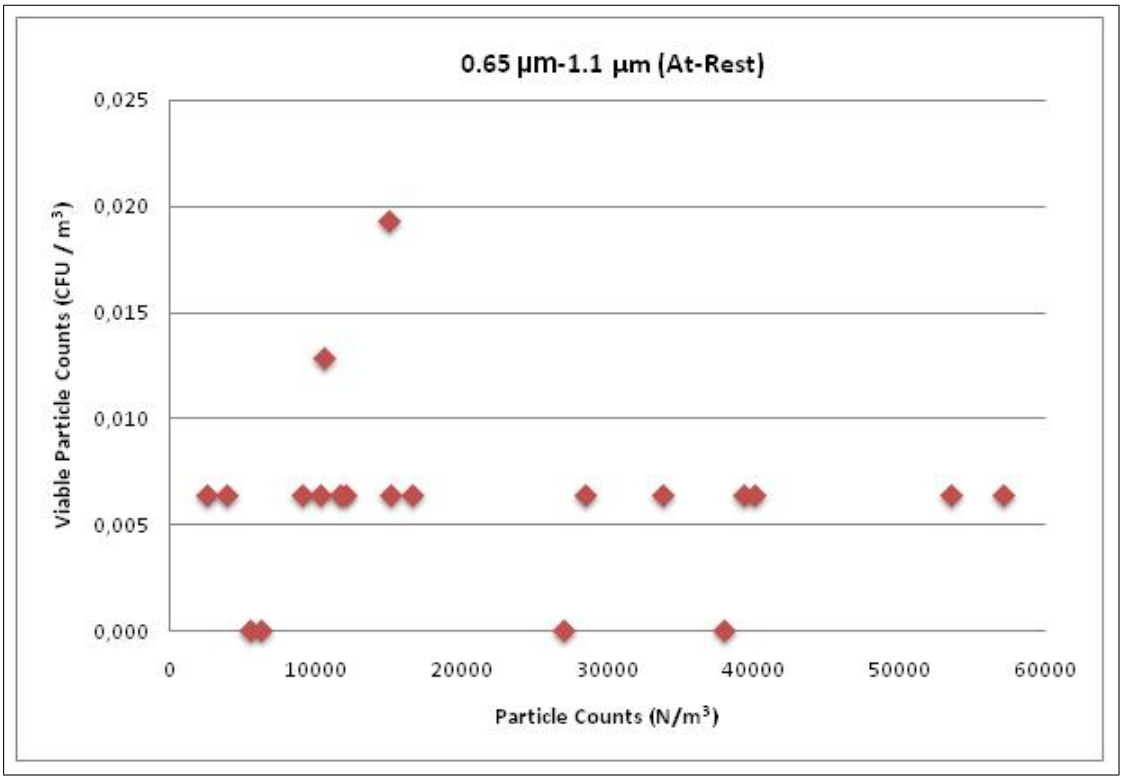

Figure 6.2 'At-Rest' measurements for the size range 0.65 μm - 1.1 μm

follow a normal distribution.

As data are not normally distributed, Spearman's correlation coefficient is used for the assessment of correlation between Particle Counting and Microbiological Sampling [8].

The correlation coefficients (RHO) and p values of the measurements with respect to the size ranges are listed in Table 6.6 and Table 6.7.

The Wilkinson Signed Rank Test is performed for testing the correlation or difference between the air quality monitoring measurements done before the operation (At Rest) and during the surgery. Results of the Wilkinson Signed Rank Test are shown at Table 6.8.

As it is not possible to obtain a statistically significant result in 'size-by-size' comparison, a different approach is applied. In this formulation, the particle counts in

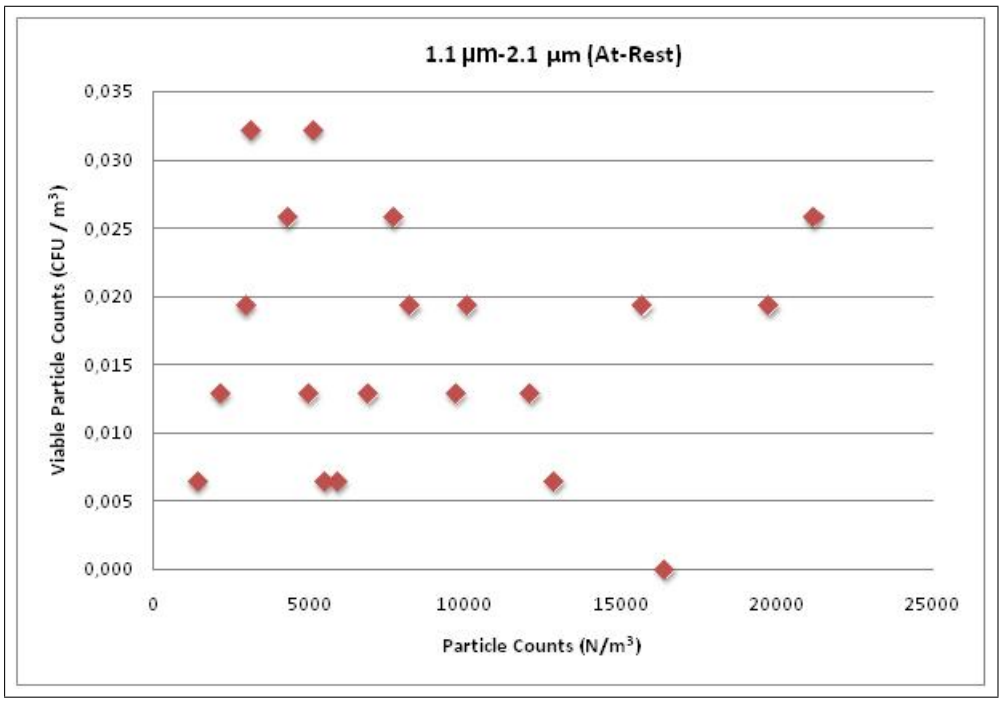

Figure 6.3 'At-Rest' measurements for the size range 1.1 μm - 2.1 μm

Table 6.3 and Table 6.4 are correlated with total number of viable particles without taking into account of the particle sizes. By doing so, one can give the answer of the following question : In particle counting, do any size range have a correlation with the total bacteria amount ? Or, perhaps particles of what size range are likely to be bacteria carrying particles? Seal and Clark worked on this approach and they found a good correlation for particles ranging between 5.0 μm and 7.0 μm [14]. The results of the Spearman's correlation coefficient test are given in Table 6.9 and Table 6.10. Particle counts and total viable counts are plotted in 'At-Rest' (Figure 6.10, Figure 6.11, Figure 6.12, Figure 6.13, Figure 6.14, Figure 6.15) and "During Surgery conditions" (Figure 6.16, Figure 6.17, Figure 6.18, Figure 6.19, Figure 6.20, Figure 6.21). Viable particles of the size 4.7 μm - 7.1 μm and $\geq$ 7.1μm are not detected.

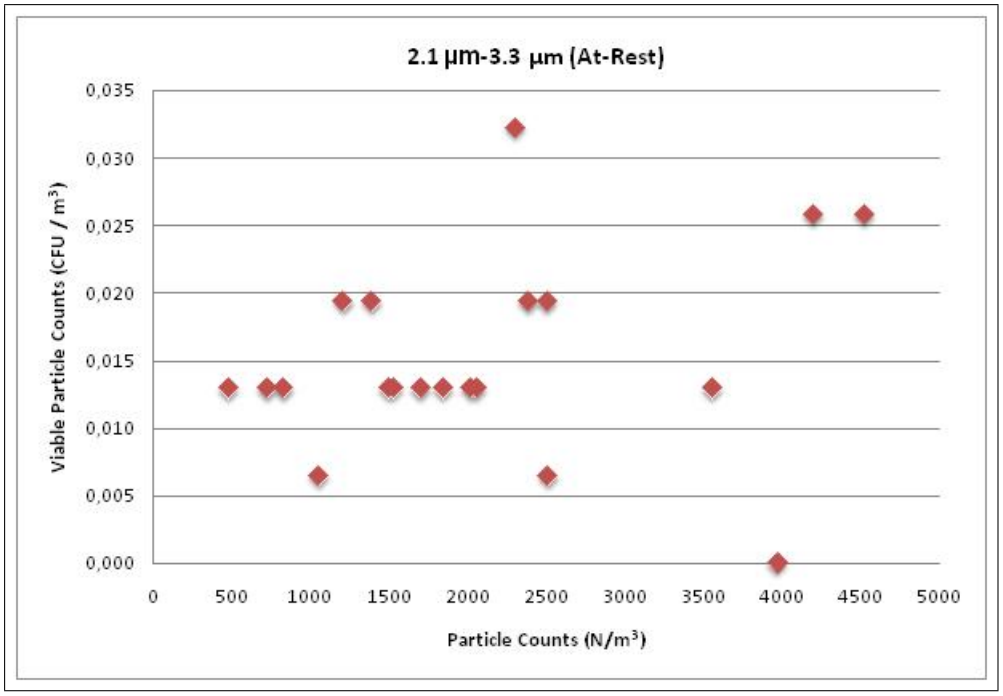

Figure 6.4 'At-Rest' measurements for the size range 2.1 μm - 3.3 μm

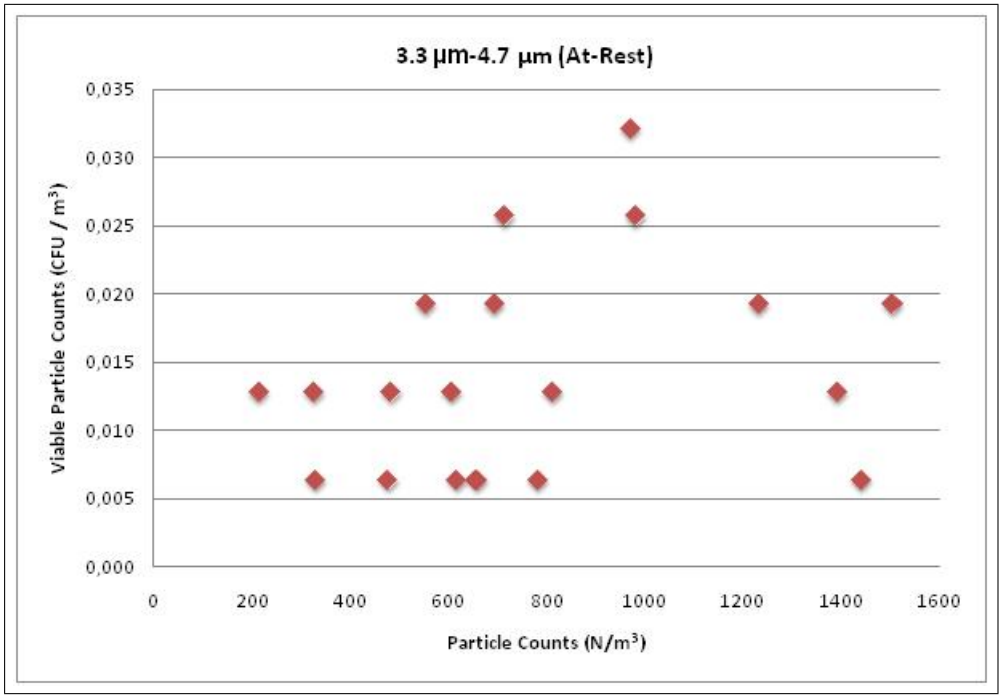

Figure 6.5 'At-Rest' measurements for the size range 3.3 μm - 4.7 μm

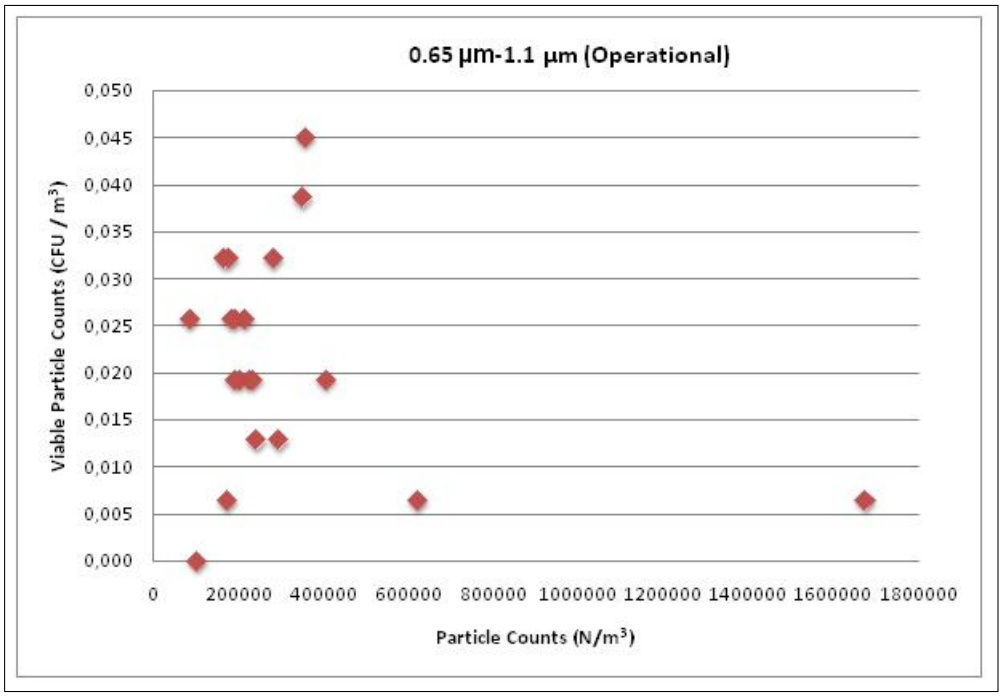

Figure 6.6 'During Surgery' measurements for the size range 0.65 μm - 1.1 μm

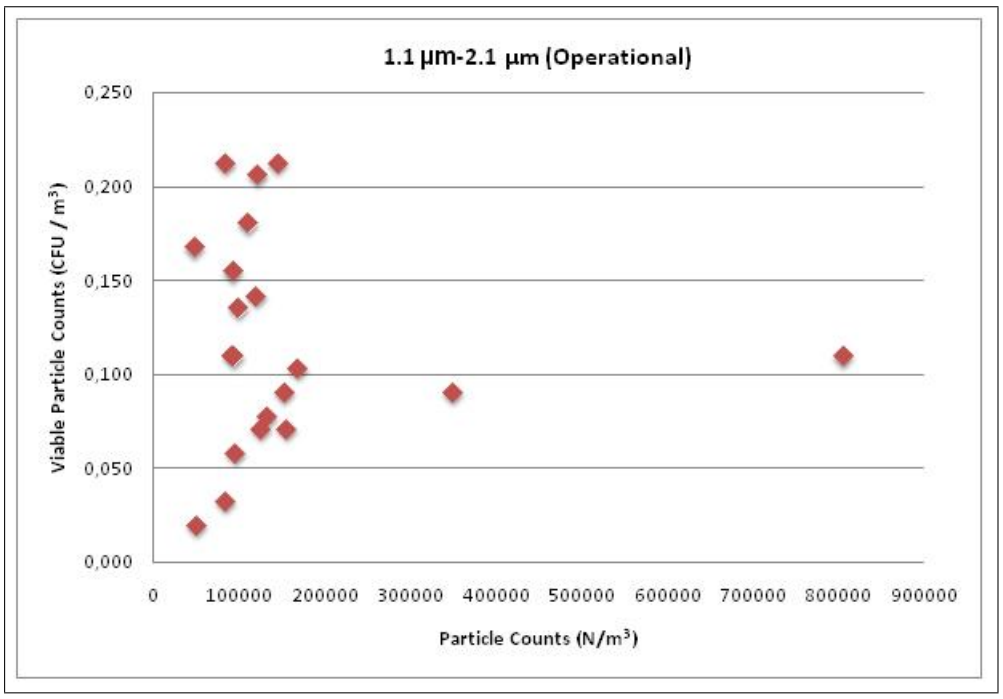

Figure 6.7 'During Surgery' measurements for the size range 1.1 μm - 2.1 μm

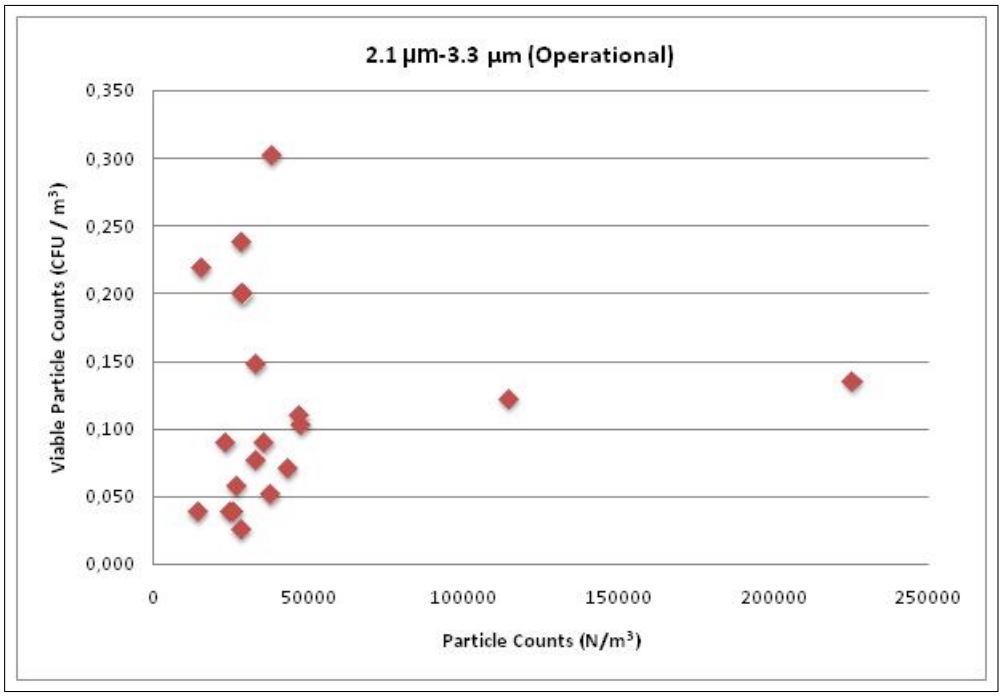

Figure 6.8 'During Surgery' measurements for the size range 2.1 μm - 3.3 μm

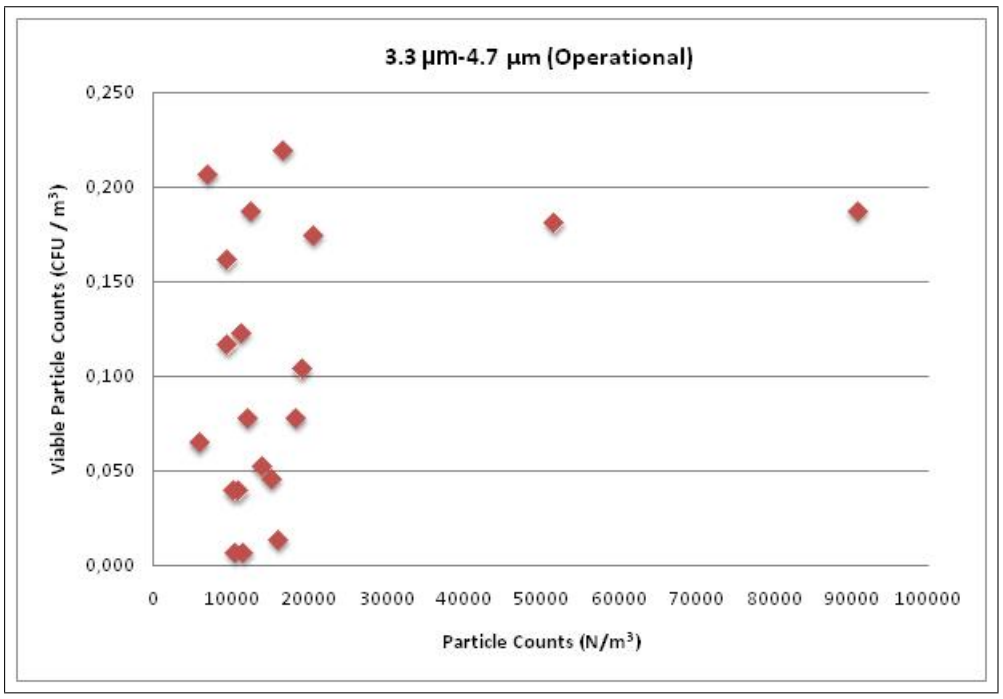

Figure 6.9 'During Surgery' measurements for the size range 3.3 μm - 4.7 μm

Table 6.5
Result of Lillie Normality Test.

Lillie Normality Test			
	h	p	ks
Particle Counter At-Rest	1	$\succ 0.20$	0.30
Viable Sampler At-Rest	1	$\succ 0.20$	0.22
Particle Counter During Surgery	1	$\succ 0.20$	0.32
Viable Sampler During Surgery	1	$\succ 0.20$	0.20

Table 6.6
Result of Spearman's Correlation Test for 'At-Rest' condition.

At-Rest		
Particle Size Range	RHO	p
0.65-1.1 μm	0.04	0.85
1.1-2.1 μm	-0.04	0.85
2.1-3.3 μm	0.23	0.32
3.3-4.7 μm	0.32	0.16
4.7-7.1 μm	NaN	1.00
$\geq 7.1\mu m$	NaN	1.00

Table 6.7
Result of Spearman's Correlation Test for 'During Surgery' condition.

During Surgery		
Particle Size Range	RHO	p
0.65-1.1 μm	-0.08	0.74
1.1-2.1 μm	-0.07	0.78
2.1-3.3 μm	0.28	0.22
3.3-4.7 μm	0.25	0.29
4.7-7.1 μm	NaN	1.00
$\geq 7.1\mu m$	NaN	1.00

Table 6.8
Result of Wilkinson Signed Rank Test.

Wilkinson Signed Rank Test		
Condition	h	p
Particle Counting At-Rest - Operational	1	$\prec 0.01$
Microbiological Sampling At-Rest - Operational	1	$\prec 0.01$

Table 6.9
Results of Spearman's Correlation Coefficient Test between total particle counts and total viable particles for 'At-Rest' condition.

At-Rest		
Particle Size Range	RHO	p
0.3-0.5 μm	0.06	0.80
0.5-1.0 μm	0.16	0.50
1.0-5.0 μm	0.14	0.57
5.0-10.0 μm	0.52	0.02
10.0-25.0 μm	0.51	0.02
$\geq 25.0\mu$m	0.15	0.51

Table 6.10
Results of Spearman's Correlation Coefficient Test between total particle counts and total viable particles for 'During Surgery' condition.

At-Rest		
Particle Size Range	RHO	p
0.3-0.5 μm	-0.17	0.47
0.5-1.0 μm	0.48	0.03
1.0-5.0 μm	0.56	0.01
5.0-10.0 μm	0.11	0.66
10.0-25.0 μm	0.22	0.36
$\geq 25.0\mu$m	0.45	0.05

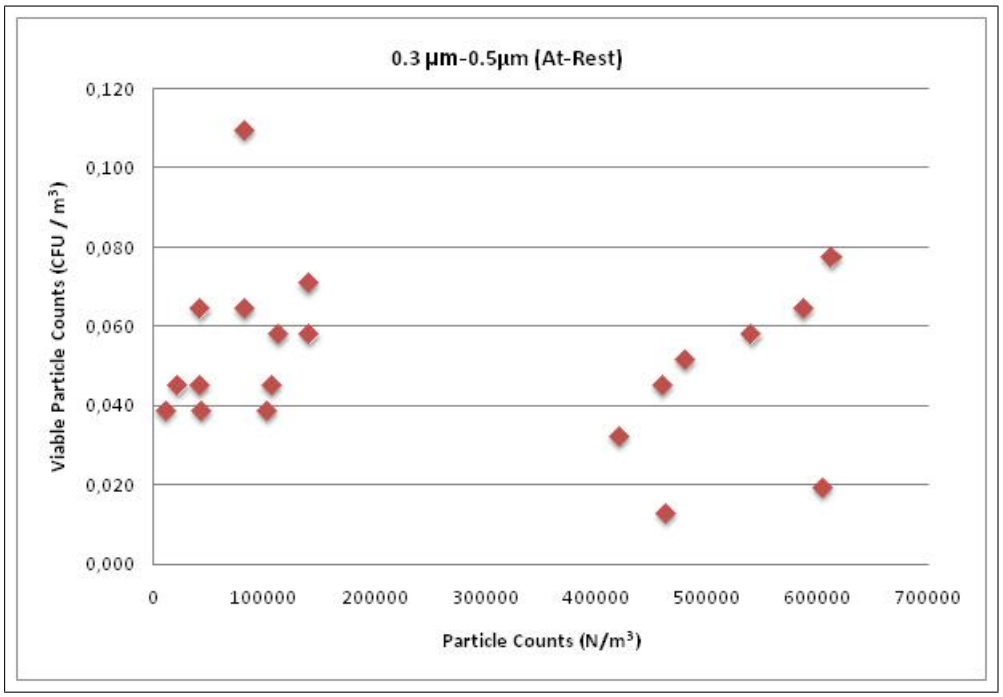

Figure 6.10 Total bacteria counts vs. particle counts of the size range 0.3 μm - 0.5 μm ('At-Rest').

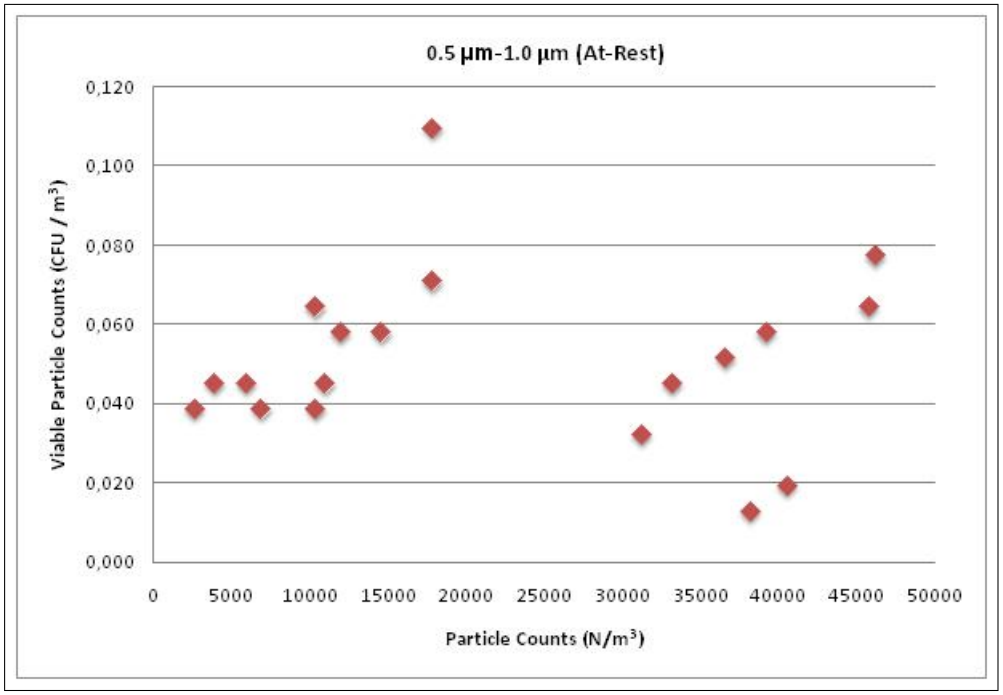

Figure 6.11 Total bacteria counts vs. particle counts of the size range 0.5 μm - 1.0 μm ('At-Rest').

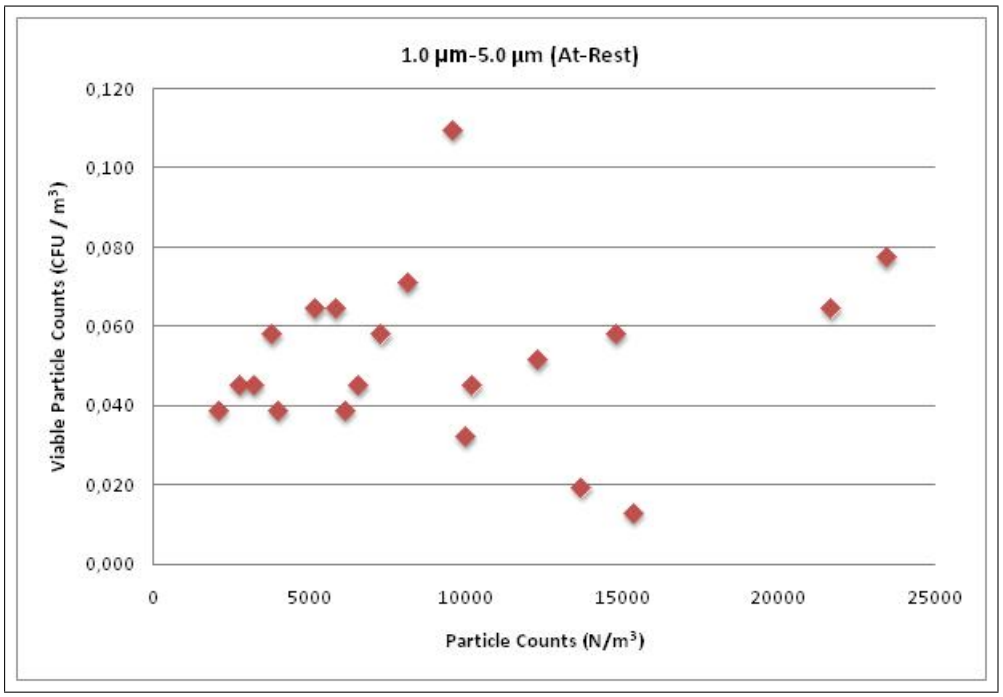

Figure 6.12 Total bacteria counts vs. particle counts of the size range 1.0 μm - 5.0 μm ('At-Rest').

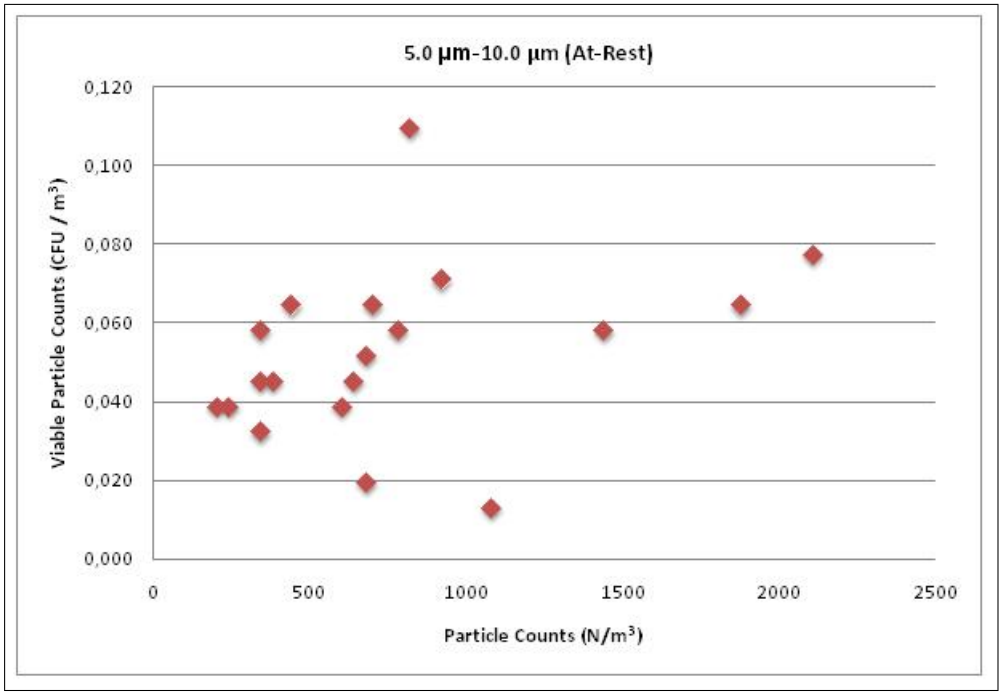

Figure 6.13 Total bacteria counts vs. particle counts of the size range 5.0 μm - 10.0 μm ('At-Rest').

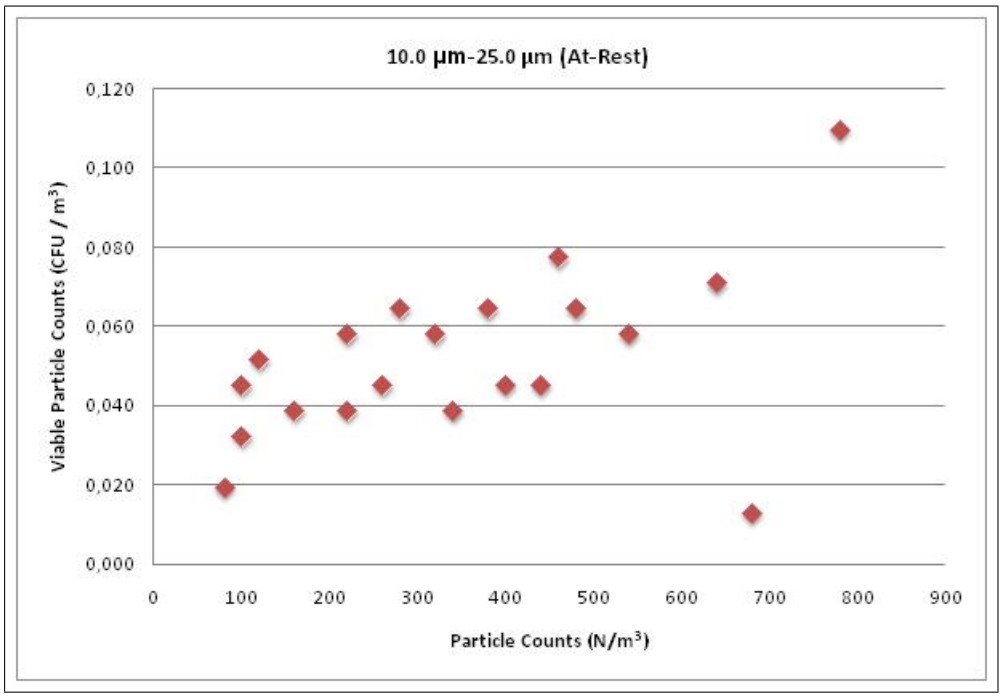

Figure 6.14 Total bacteria counts vs. particle counts of the size range 10.0 μm - 25.0 μm ('At-Rest').

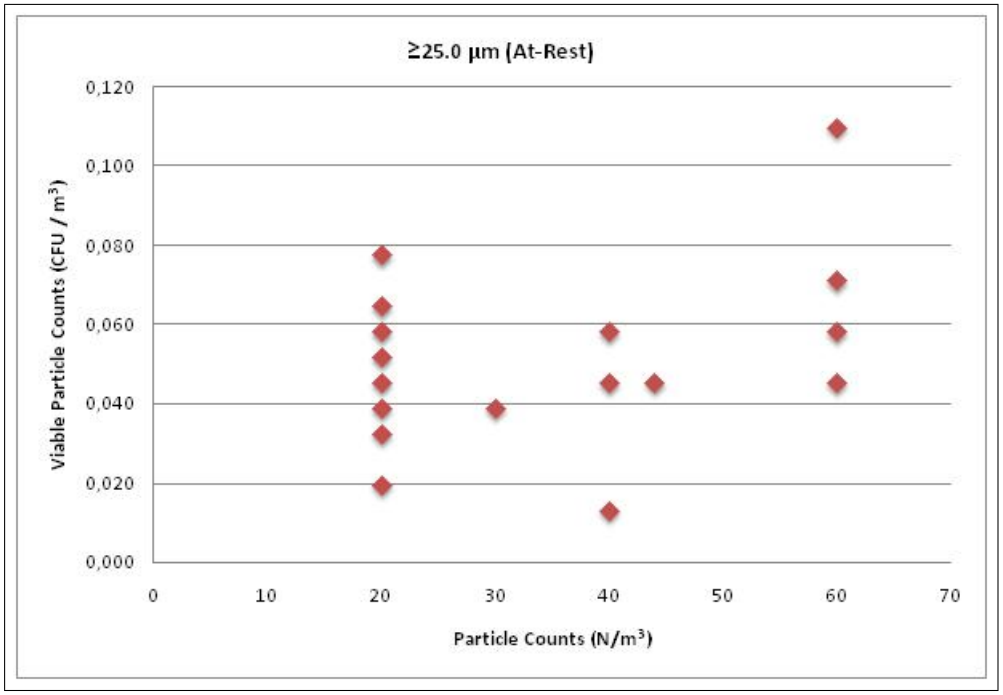

Figure 6.15 Total bacteria counts vs. particle counts of the size range $\geq$ 25.0μm ('At-Rest').

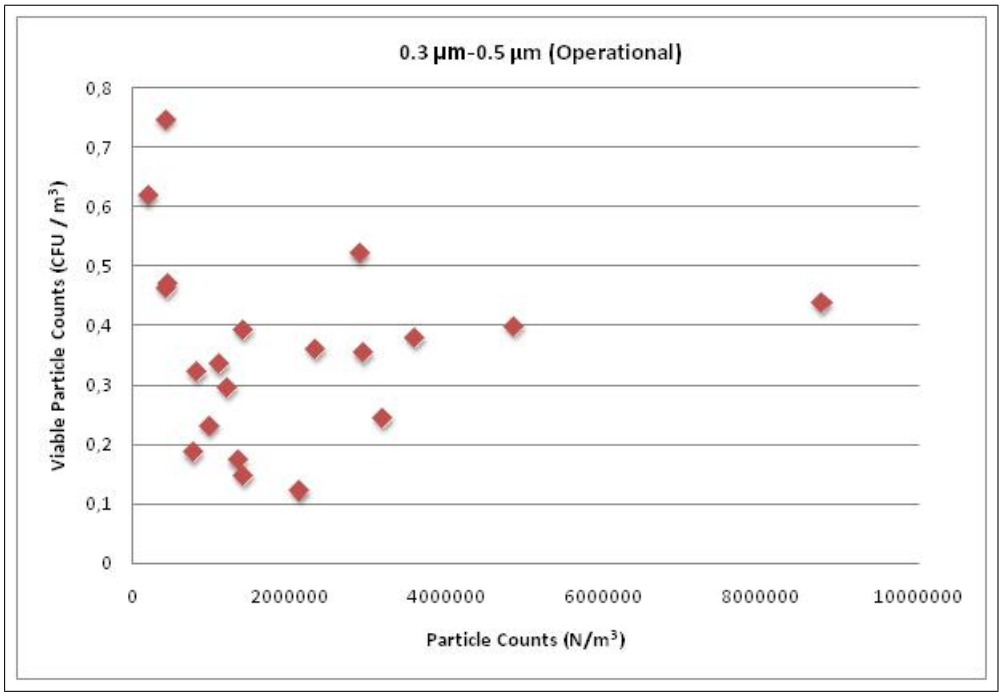

Figure 6.16 Total bacteria counts vs. particle counts of the size range 0.3 μm - 0.5 μm ('During Surgery').

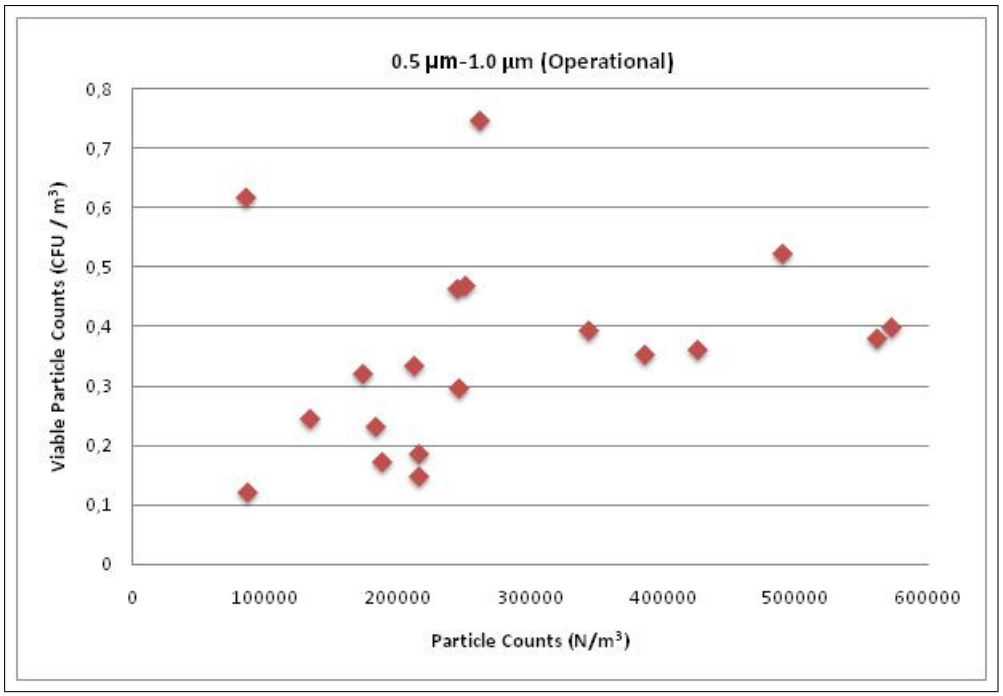

Figure 6.17 Total bacteria counts vs. particle counts of the size range 0.5 μm - 1.0 μm ('During Surgery').

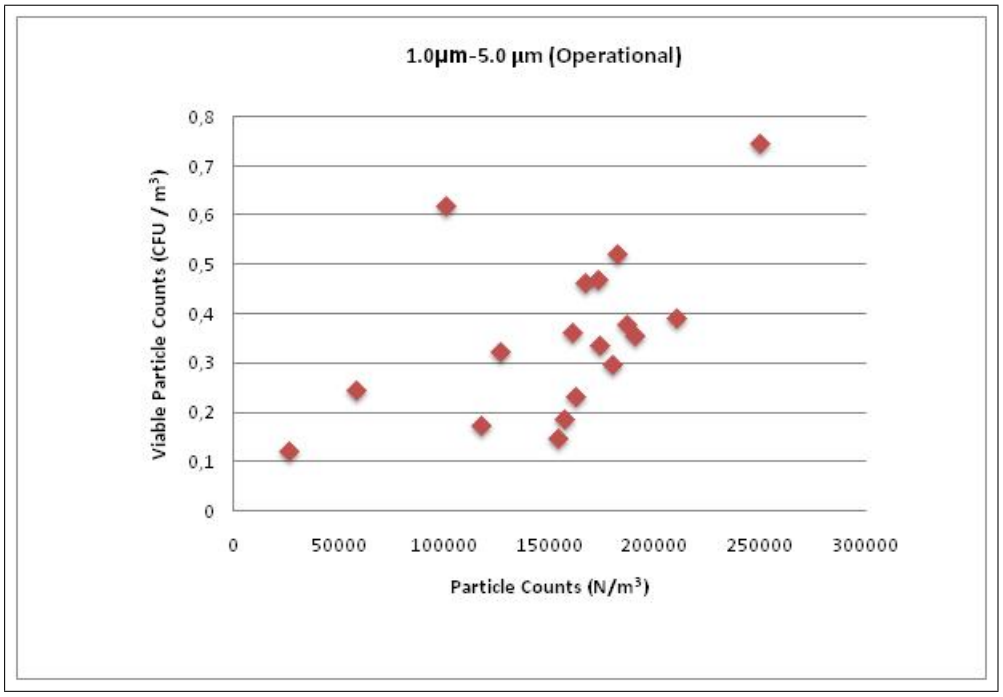

Figure 6.18 Total bacteria counts vs. particle counts of the size range 1.0 μm - 5.0 μm ('During Surgery').

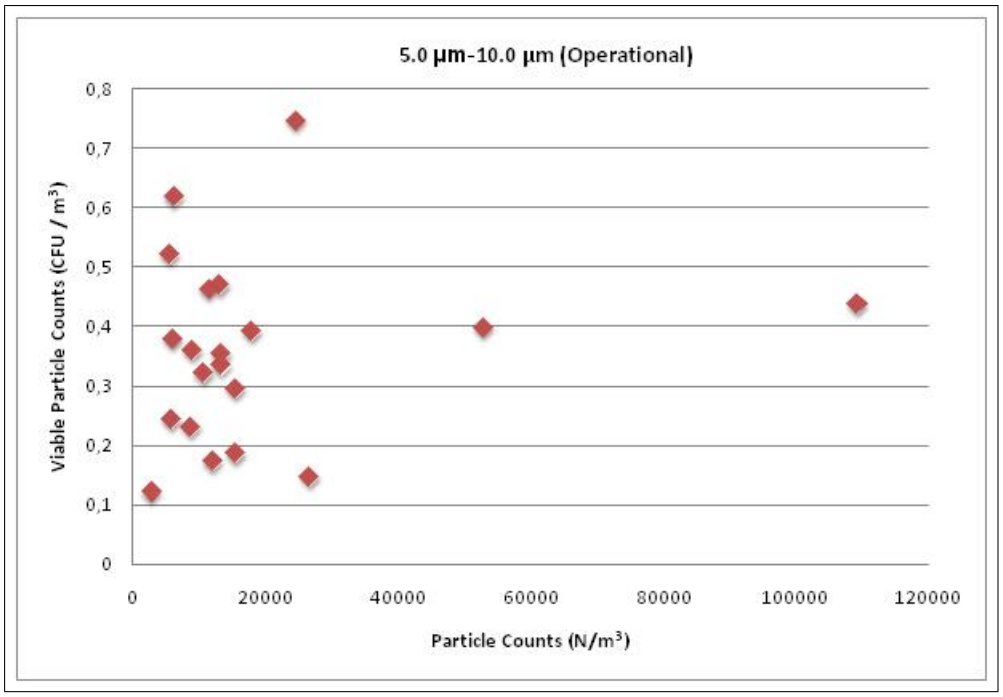

Figure 6.19 Total bacteria counts vs. particle counts of the size range 5.0 μm - 10.0 μm ('During Surgery').

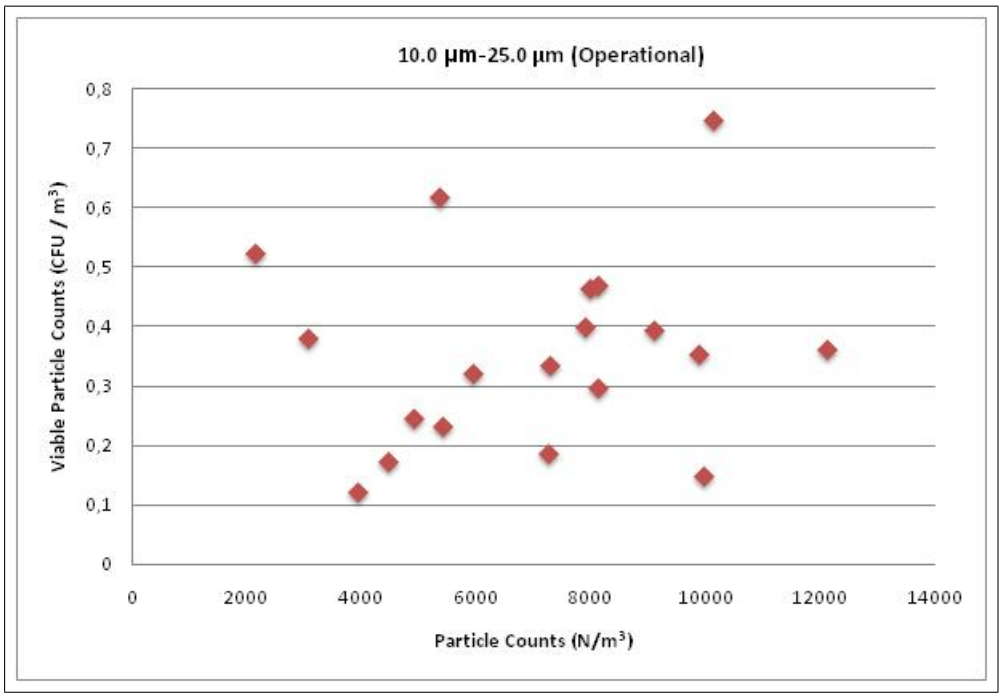

Figure 6.20 Total bacteria counts vs. particle counts of the size range 10.0 μm - 25.0 μm ('During Surgery').

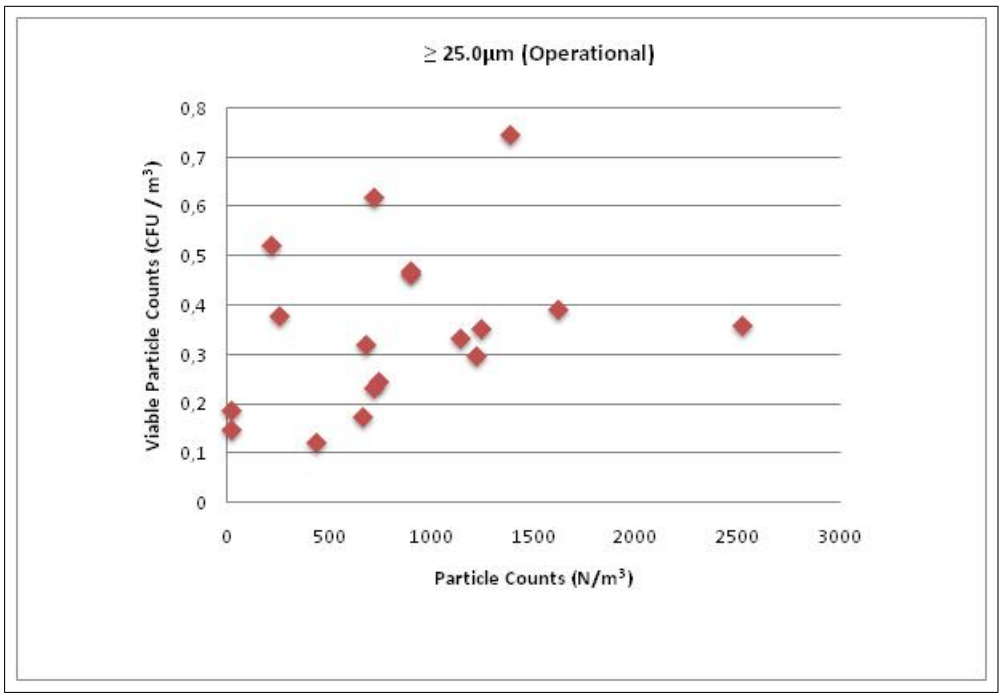

Figure 6.21 Total bacteria counts vs. particle counts of the size range $\geq$ 25.0μm ('During Surgery').

7. CONCLUSION

In the past, measurements are statistically evaluated by other researchers to test if particle counting can replace microbiological sampling; however, the results do not confirm such a correlation [8]-[14]. They used particle counters to range the size of particles, but they did not use a range-sizing equipment for detecting viable microorganisms, they counted total bacteria carrying particles and compared them with particle sizes.

In this study, the New-Star Andersen Six-Stage Viable Counter is used for microbiological sampling to classify the particles into size ranges similarly to particle counters. So, the bacteria amount can be discriminated into different size ranges and then compared with the result of particle counting results.

Spearman's Correlation Coefficient Test (Table 6.6, Table 6.7) showed that no correlation can be found in any of the size ranges between the Particle Counting and Microbiological Sampling neither in 'at-rest' condition, nor during surgery. None of the RHO coefficients could approximate to -1 or 1.

In microbiological sampling, Sabouraud's Dextrose Agar is used at Stage 1 and Stage 2 to detect fungal spores. However, in none of the measurements, type of fungi is detected. A control experiment is made by using Sheep Blood Agar %5 at Stage 1 and 2, but there has been no significant change. HEPA Filters used in operating room ventilation significantly prevent the presence of fungi [43]. In all of the five operating rooms, HEPA filters are used. So, no fungi detection can be called as a normal result.

Since size-by-size comparison did not give any correlation between the two methods, another approach used by Seal and Clark used is considered [14]. This approach gave significantly good results, although pre-operational measurements and operational measurements gave different correlations. For 'At-Rest' measurements, there is a sig-

nificant relationship between particles in the size ranges 5.0-10.0 μm and 10.0-25.0 μm (Table 6.9) and total viable particle counts which confirms Seal and Clark who say that this relation happens at 5.0 μm -7.0 μm range [14]. In measurements taken during surgery, there is a significant relationship between particles in the size ranges 0.5-1.0 μm, 1.0-5.0 μm and $\geq$ 25.0μm and total viable particle counts (Table 6.10). When we combine rest and operational conditions combined together, there exists a correlation between particle counting and microbiological sampling for particles larger than 0.5 μm which most of the bacteria stay in this size range.

In the experiment design stage, we thought that experiments should not only be made at-rest condition, but also we should make the experiment during the operations by thinking the fact that number of particles and bacteria counts will increase, so we would have different and more polluted type of data[15]. From Table 6.8, Wilkinson Signed Rank Test confirms us; during the operation, air quality monitoring done by particle counting and microbiological sampling methods is totally different from at-rest mode. Both Particle Amount detected by particle counter and viable aerosol amount significantly increased at operational measurements.

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