European Journal of Parenteral & Pharmaceutical Sciences 2017; **22**(4): 126-138

Calculation of air supply rates and concentrations of airborne contamination in non-UDAF cleanrooms

W Whyte[1], T Eaton[2], WM Whyte[1], N Lenegan[3], S Ward[4] and K Agricola[5]
[1] *School of Engineering, University of Glasgow, Glasgow G12 8QQ*
[2] *AstraZeneca, Macclesfield, Cheshire, SK10 2NA*
[3] *Energy and Carbon Reduction Solutions, Ashton-Under-Lyne, Lancashire, OL5 0RF*
[4] *Validair, Wrotham, Kent, TN15 7RJ*
[5] *Technology of Sense BV, Institutenweg 15, Enschede, 7521PH, the Netherlands*

This article reviews a series of scientific articles written by the authors, where the following topics were investigated in relation to non-unidirectional airflow cleanrooms.
(1) The air supply rate required to obtain a specified concentration of airborne contamination.
(2) The calculation of concentrations of airborne contaminants in different ventilation and dispersion of contamination situations.
(3) The decay of airborne contamination
(a) during the 'clean up' test described in Annex 1 of the EU Guidelines to Good Manufacturing Practice (2008);
(b) during the recovery rate test described in Annex B12 of ISO 14644-3 (2005);
(c) associated with clean areas, such as airlocks, to reduce airborne contamination before a door into a cleanroom is opened.
Worked examples are provided to demonstrate the calculation methods to provide solutions to the above topics.

Key words: Air supply rates, airborne concentration, airborne contamination, particles, MCPs, cleanrooms, ventilation equations, decay of contamination.

Introduction

This article contains a review of seven articles written by the authors on the topics listed in the abstract, along with two articles about deposition of airborne contamination onto cleanroom surfaces that causes reduction in airborne particle concentration. These nine articles are listed in order of the date of publication at the end of this article and referenced in the text, as follows, by use of Roman numerals: 'Article I'. Other articles are referenced by means of a superscript number and listed as additional references.

When designing a non-unidirectional airflow (non-UDAF) cleanroom to achieve a required airborne cleanliness, such as specified in ISO 14644-1 (2015)[1], Annex 1 of the European Union Guidelines to Good Manufacturing Practice[2] (EU GGMP), or the US Food and Drug Administration – Current Good Manufacturing Practice (2004)[3], designers have to decide how much filtered air should be supplied. This decision is normally based on experience and 'rules of thumb' and not by an analytical method. The consequence is that many cleanrooms have excessive air supply that is associated with high capital and running costs, and energy waste. Conversely, a low air supply may result in too high a concentration of contamination, and major remedial work to rectify the problem. It would be useful if an analytical method was available to calculate the air supply rate, as well as clarifying what variables affected the calculation, and their relative importance.

Several scientific articles have been written about methods of calculating the expected concentrations of airborne contamination in cleanrooms[4–10], and the equations in these articles can be adapted to calculate the air supply rate for a specified airborne concentration of contamination.

The decay of airborne contamination in non-UDAF cleanrooms is associated with the 'clean up' test suggested in Annex 1 of the EU GGMP (2008)[2], the recovery rate test in Annex B12 of ISO 14644-3 (2005)[11], and ventilation requirements for clean areas, such as airlocks. These three requirements can be achieved from knowledge of the

***Corresponding author:** Tim Eaton, Sterile Manufacturing Specialist, AstraZeneca, UK Operations, Silk Road Business Park, Macclesfield, Cheshire, SK10 2NA; Email: tim.eaton@astrazeneca.com; Tel: +44(0)1625 514916.

relationships that govern the decay of airborne contamination in enclosed spaces, such as cleanrooms.

The build-up, steady-state, and decay of airborne contamination

To provide solutions to the situations outlined above, it is necessary to understand the three ventilation conditions that determine the concentration of particles and microbe-carrying particles (MCPs) in cleanroom air. If a cleanroom is in the 'at rest' state with no personnel present and no machinery working, there will be no airborne contamination dispersed. As the air supply to the cleanroom is filtered and essentially free of contamination, and the cleanroom pressurised to prevent contamination entering from adjacent areas, the concentration of airborne contamination in such a cleanroom should be close to zero. However, when personnel enter and operations start, the dispersion of airborne particles and MCPs causes their concentration to 'build up' to a plateau, or 'steady-state' condition. In the 'steady-state' condition, the contamination dispersed into air equals that removed by ventilation, and the concentration remains relatively steady. When contamination-generating activities cease, or decrease, the airborne concentration of contamination will 'decay'. The three conditions of 'build-up', 'steady-state' and 'decay' are illustrated in **Figure 1**.

Calculation of the 'build-up' of airborne contamination is of limited use in cleanrooms, but knowledge of 'steady-state' and 'decay' concentrations is useful; particularly the 'steady-state', which determines the average airborne concentration during operation and, therefore, the amount that may deposit onto a product. The concentrations of airborne contamination in the steady-state and decay conditions can be calculated by equations described in the next section.

The ventilation equations

Equations are used in conventional mechanically ventilated rooms, such as offices, to predict toxic gaseous contamination in the 'build-up', 'steady-state' and 'decay' conditions. These equations are discussed in Article I, which reports their modification for use in cleanrooms. The equations only apply to non-UDAF cleanrooms, as the derivation of the equations is based on the assumption that room air is well mixed, and this assumption does not apply to UDAF conditions, where contamination is displaced by a piston of contamination-free air.

Steady-state equations

Equation 1 is a simple dilution equation used to estimate the airborne concentration of contaminants in a non-UDAF cleanroom in the 'steady-state' condition, when the air supply rate to the cleanroom and dispersion rate of airborne contamination are known. It is applied in this article to the most common sizes of airborne particles used to classify and monitor cleanrooms, i.e. ≥0.3 µm, ≥0.5 µm and ≥5 µm, as well as to MCPs.

Equation 1

$$C = \frac{D}{Q}$$

Where,
C = concentration of airborne contamination
D = dispersion rate of airborne contamination
Q = air supply rate into cleanroom

Alternatively, the air supply rate into a cleanroom *(Q)* required for a specified concentration of airborne contamination can be calculated by Equation 2.

Equation 2

$$Q = \frac{D}{C}$$

Equations 1 and 2 do not include several factors present in cleanrooms that are likely to affect the calculations. These are discussed in Article IV, and are as follows.

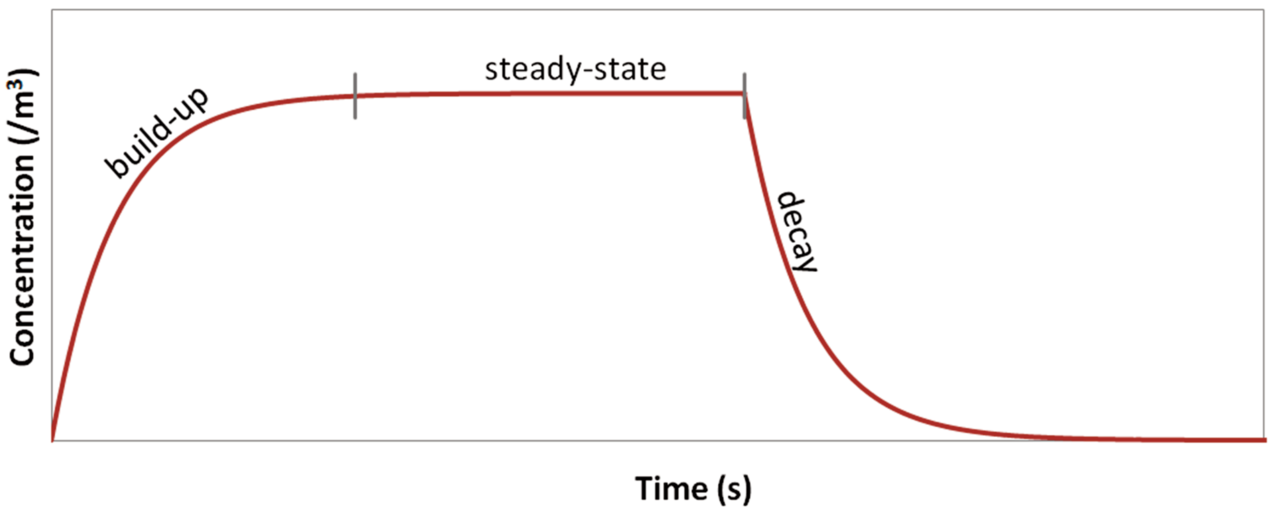

Figure 1. Build-up, steady-state and decay of airborne contamination in a ventilated room.

1. The ventilation effectiveness in a non-UDAF cleanroom may vary across a cleanroom, and give locations with higher than average concentrations of contamination. To compensate for this, the increase of the air supply rate is calculated by the use of a ventilation effectiveness index *(ε)*, as shown in Equation 3.

Equation 3

$$Q = \frac{D}{\varepsilon C}$$

2. The airborne concentration of contaminants in non-UDAF cleanroom will be reduced by air contributed from clean air devices, such as in UDAF zones and workstations, open restricted access barrier systems (RABS), or isolators, installed in the cleanroom. However, only a proportion of the air from a device will mix effectively with the cleanroom air and a ventilation efficiency coefficient (β) should be included in the calculation.
3. The deposition of particles onto cleanroom surfaces will reduce the airborne concentration. The surface deposition of particles of ≥0.3 µm and ≥0.5 µm is low and need not be included (see *Surface deposition of particles and MCPs* section). However, microbes are carried on skin and clothing particles, and have an average aerodynamic equivalent diameter of about 12 µm, and readily deposit onto cleanroom surfaces by gravity. Therefore, if MCPs and large particles ≥5 µm are considered, the reduction by surface deposition should be included.

The three additional variables listed above are included in the following Equation 4 to calculate the air supply rate for a required concentration of airborne contamination. Should a clean air device not be installed, the second term in the equation can be disregarded, and if the surface deposition is not included the third term can be disregarded.

Equation 4

$$Q = \frac{D}{\varepsilon C} - \beta Q_D - V_D A$$

Where
Q = required air supply rate (m^3/s)
D = average value of dispersion rate (no./s)
ε = ventilation effectiveness index
C = concentration of airborne contamination (no./m^3)
β = ventilation efficiency coefficient of clean air device
Q_D = air supply rate passing through clean air device (m^3/s)
V_D = deposition velocity of MCPs and particles passing through air and onto a surface (m/s)
A = surface area of a cleanroom where deposition occurs – usually equivalent to the floor area (m^2)

If the airborne concentration of contamination has to be calculated, then Equation 5 can be used.

Equation 5

$$C = \frac{D}{\varepsilon(Q + \beta Q_D - V_D A)}$$

It should be noted that in Equations 4 and 5, the ε and β variables may interact, and this should be considered (see *Worked examples of the calculation of air supply rates or airborne concentrations in a non-UDAF cleanroom* section). It should also be noted that Equations 1 to 5, show that to calculate either the airborne concentration of contamination, or the required air supply rate to a non-UDAF cleanroom in the 'steady state' condition, it is necessary to use the **air supply rate**, and not the **air change rate**. This is discussed in Article I and illustrated by the following practical example.

Consider a small cleanroom with a floor area of 6 m x 5 m and ceiling height of 3 m, i.e. a room volume of 90 m^3. If the air change rate is 20 per hour then the air supply rate would be 90 x 20 = 1800 m^3/h. However, if the room was twice the original size, i.e. 180 m^3, then to obtain the same air change rate of 20/h, it would require twice the air supply rate, i.e. 3600 m^3/h. In this situation, there would be twice the air supplied to dilute and remove contamination, and hence the airborne concentration of contamination in the larger room will be half the concentration in the smaller room for the same cleanroom air change rate.

Decay equation

When dispersion of contamination ceases in a non-UDAF cleanroom, the airborne contamination will decay exponentially in the manner shown in **Figure 2**, which shows the decay in three non-UDAF cleanrooms with different air change rates. Cleanrooms with the same air change rates will have the same decay rate, irrespective of the air supply rate. If the contaminant is not removed by deposition onto surfaces, as occurs with gases and small particles, the concentrations of airborne contamination during decay can be calculated by the following equation.

Equation 6

$$C = C_o \cdot e^{-Nt}$$

Where,
C = concentration of contamination after time t
C_o = initial concentration
N = air change rate in the cleanroom
t = elapsed time

It should be noted that Equation 6 shows that the decay rate is dependent on the **air change rate**, and not the **air volume supply rate**. Although the air supply rate must be used to calculate the airborne concentration of contamination in the steady-state condition, the air change rate must be used to calculate decays of airborne contamination associated with recovery rates for cleanrooms, or clean areas such as airlocks.

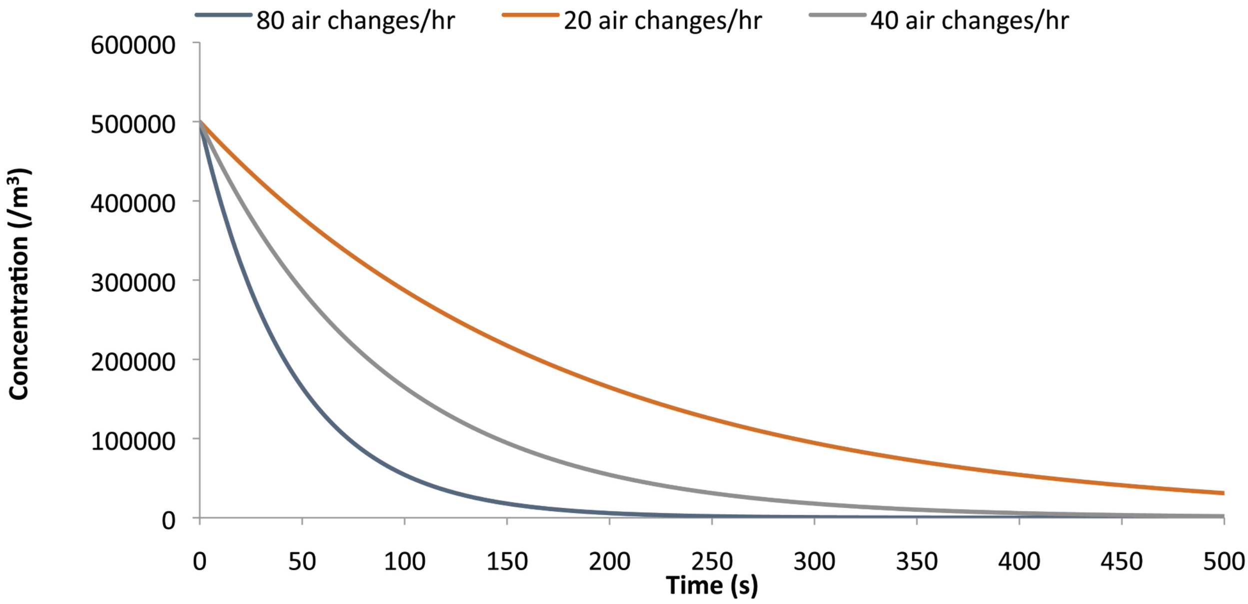

Figure 2. Decay of airborne contamination in cleanrooms with different air change rates.

Application of the decay equation

The application of the decay Equation 6 is considered in Article II, where it is explained that Equation 6 can be rewritten so that the air change rate *(N)* at a location can be obtained from the rate of decay of airborne contamination. This equation can be given in natural logarithms, or logarithms to the base 10, and is as follows.

Equation 7

$$N = -\frac{1}{t}\ln\frac{C}{C_0} = -2.3 \times \frac{1}{t}\log_{10}\frac{C}{C_0}$$

The cleanroom standard, ISO 14644-3 (2005)[11] contains a test that is used to obtain the cleanliness 'recovery rate' and calculated by Equation B12 that is given in the standard, and is as follows.

$$n = -2.3 \times \frac{1}{t_1}\log_{10}\frac{C_1}{C_0}$$

Where,

n = recovery rate

t_1 = time elapsed between the first and second measurement

C_0 = initial concentration

C_1 = concentration after time t_1

If the measurement units are the same, e.g. number of air changes/hour, it is seen that the right-hand side of Equation B12 is the same as the right-hand side of Equation 7 and, therefore, the 'recovery rate (n)' is the same as the 'air change rate (N)', when measured at the same location.

The cleanliness recovery test described in ISO 14644-3 (2005)[11] fails to suggest what recovery rate results are acceptable, or not. However, if the air change rate at a location obtained from measuring the recovery rate is compared with the average air change rate of the entire cleanroom, a ventilation effectiveness index known as the Air Change Effectiveness (ACE) is obtained by Equation 8. If the ACE index is calculated, then a useful numerical result will be obtained to establish whether the recovery rate at the measuring location is higher or lower than average. Further information about the ACE index is given in the *Ventilation effectiveness* section.

Equation 8

Ventilation effectiveness index (ACE)

$$= \frac{\text{air change rate at measuring location}}{\text{average air change rate in cleanroom}}$$

Articles II and III discuss the EU GGMP Annex 1 (2005)[2] 'clean-up' test required in pharmaceutical cleanrooms to demonstrate that when production ceases the particle concentration quickly decays to the 'at rest' condition. By use of the decay equation, the number of air changes required to ensure that a non-UDAF cleanroom complies with the 'clean-up' test requirements can be obtained. This calculation is discussed in more depth in Article VIII, and applied in the *Worked examples of the calculation of air supply rates or airborne concentrations in a non-UDAF cleanroom* section. Finally, in the design of air locks, suitable air change rates and time delays before doors are opened can be calculated, so that undesirable transfer of airborne contamination across the airlock is minimised; this information is included in Article II.

Information required for the calculation of air supply rates and airborne concentrations

To calculate either the air supply rate, or concentration of contaminants in a non-UDAF cleanroom, Equations 1 to 5 can be used. However, values of the equation variables are required, and these are now considered.

Dispersion rates from personnel and machinery

To calculate the required air supply rate, or the airborne concentration in a non-UDAF cleanroom, the dispersion rates of airborne contamination from sources such as personnel and machines is required. All individual dispersion rates should be added together to obtain the total dispersion rate.

If required, the dispersion rates can be obtained in a cleanroom or test chamber containing the active source, using a method similar to that suggested in Annex B4 of ISO 14644-14[13]. The test space should be supplied with a known rate of particle-free air, which pressurises the test space against the ingress of contamination from adjacent areas. If the average concentration of airborne contamination is measured in the steady-state condition, the dispersion rate can be calculated as follows.

Equation 9

Dispersion rate (no./s) = air volume supply to test space (m^3/s) × average concentration in test space (no./m^3)

Information on typical dispersion rates of personnel is discussed in Article IV, and shown in **Table 1** is the average dispersion rate of 55 different personnel when exercising in a dispersion chamber. The beneficial effect of cleanroom garments made from a woven, reusable, polyester fabric with a pore diameter[14] of 28 µm is shown in comparison to normal indoor clothing. It was also reported that gowns (smocks) do little to reduce the dispersion rate, as much of the person's contamination is dispersed from under the gown and into the cleanroom air and, therefore, their dispersion rate can be assumed to be similar to normal indoor clothing.

Article IV gives further information on dispersion rates from personnel exercising in a dispersion chamber when wearing more effective cleanroom clothing made from a tighter-woven polyester fabric with a pore diameter of 12 µm. It can be seen in **Table 2** that much lower dispersion rates are obtained from the better clothing. When wearing the same clothing within an operational cleanroom, where personnel activity was lower than exercising in a dispersion chamber, the lower activity gave even lower dispersion rates.

Shown in **Table 3** is information given in Article IV of emission rates of particles from various types of machinery.

Ventilation effectiveness

When filtered air is supplied to a non-UDAF cleanroom, the air movement pattern in the room may result in less clean air than the average reaching a critical location. To compensate for this, an increase in the air supply is required, and this can be calculated by use of a ventilation effectiveness index and Equations 3 or 4.

A large number of types of ventilation effectiveness indexes exist, and the most commonly-known are: air change effectiveness, air change efficiency, and contamination removal effectiveness. It is considered that the ACE index is most appropriate for the design of cleanrooms[18] and also has the advantage of being obtained from the results of tests that are routinely carried out in cleanrooms (see *Application of the decay equation* section).

The ACE index is commonly used in ordinary ventilated rooms, such as offices, and described in ANSI/ASHRAE standard 129-1997 (RA 2002)[12]. The air change efficiency index is described in the REHVA

Table 1. Average dispersion rate of particles and MCPs from people exercising in a test chamber.

Type of cleanroom garments	Average dispersion rate per person (counts/s)		
	Particles		MCPs
	≥0.5 µm	≥5 µm	
Normal indoor clothing	35,500	5500	40
Typical coveralls, hood and full-length boots	17,000	600	3

Table 2. Average dispersion rate of particles and MCPs from people exercising in a test chamber, or working in an operational cleanroom.

Type of activity	Dispersion from one person/s		
	≥0.5 µm	≥5 µm	MCPs
Exercising in dispersal chamber	2170	550	0.13
Normal activity in cleanroom	908	46	0.017

Table 3. Particle emission rates from machinery.

Type of machine or equipment	Source of information	Emission rate/s
Vial filling machine A	Hejab[15]	3.3×10^4/s particles ≥0.5 μm
Vial filling machine B	Hejab[15]	5×10^2/s particles ≥0.5 μm
Blow-fill-seal (BFS) machines	Sundstrom, Ljungqvist and Reinmuller[16]	Between 10^2 and 10^7 particles ≥0.5 μm/s, depending on type of BFS machinery
Six-axis robot – Unmodified – Modified to reduce emission	Hnatek[17]	 Unmodified robot: 4×10^3/s of particles ≥0.5 μm Modified robot: 0.3/s of particles ≥0.5 μm

Ventilation Effectiveness Guidebook No 2[18] and is very similar to the ACE index, but gives results that are half the value. The Contamination Removal Effectiveness index[18] measures the effectiveness of removal of contamination and is the ratio of the concentration of contamination at the exhaust compared to the average in the cleanroom. However, it may not be the best indication of the required increase of air supply when the critical location is situated between source and extract.

The ACE index can be measured at one or more locations in a room, and the main location suggested in the ANSI/ASHRAE standard is where a person breathes. However, in a cleanroom, it is best measured at a critical location, such as where product is exposed to airborne contamination.

The method of calculation given in the ASHRAE standard is as follows.

Equation 10

$$ACE = \frac{\tau_n}{A_i}$$

Where,
τ_n = nominal time constant
A_i = age of air at location, i

Both the 'age of air' and 'nominal time constant' are terms unlikely to be familiar to cleanroom designers and users, but it has been demonstrated in the annex of Article III that the ACE index can be calculated by the alternative Equation 11.

Equation 11

$$\text{ACE index} = \frac{\text{air change rate at measuring location}}{\text{overall air change of cleanroom}}$$

The air change rate at the measuring location can be measured by the decay of airborne contamination and use of Equation 7. However, as shown in the *Application of the decay equation* section, the air change rate at a location has the exact same value as the 'recovery rate' test described in ISO 14644-3 and, therefore, the recovery rate can be used as long as it uses the same units of measurement as the overall air change rate. The overall air change rate can be obtained in the routine way from knowledge of the air supply rate and the cleanroom's volume. Alternatively, the overall air change rate can be determined by measuring the decay of airborne particles at each of the room's exhausts, and obtaining an average that is weighted by the air volume rate through the exhausts.

As deduced from Equation 11, if the air mixing in the cleanroom is perfect, the ACE index will be 1. If less clean air than average reaches the measuring location, the ACE index will be below 1, and if more air reaches the location it will be above 1.

An experimental study of a non-UDAF cleanroom, and field tests of another 23 non-UDAF cleanrooms, were carried out to determine their ACE indexes, and reported in Article III. The article explains the method to obtain ACE indexes in cleanrooms, and reports that good air mixing cannot be assumed in non-UDAF cleanrooms. It also contains information on how poor mixing and low ventilation effectiveness can be minimised. The tests showed that when cleanrooms use efficient air supply diffusers and low-level extracts, the supply air will mix effectively with room air, and the ACE index is unlikely to be below 0.7, and may be close to 1; a value of 0.7 is a reasonable design choice.

It should be noted that the ACE index can be measured at one or more locations. Using one location will be satisfactory if airborne contamination mainly occurs at that location, but if contamination occurs at several locations, it may be necessary to determine the lowest ACE index.

Contribution of additional clean air from clean air devices

If a clean air device is installed in a non-UDAF cleanroom, then the device's air will enter the cleanroom and a proportion will mix with the room air and reduce the airborne contamination. This is discussed in Article IV.

Figure 3 shows a computational fluid dynamics (CFD) analysis of airflow in a non-UDAF cleanroom that contains a free-standing vertical UDAF workstation. It can be seen that a proportion of the air from the UDAF workstation returns to the device's air intake in the cleanroom and, as it does, it mixes well with the cleanroom air. However, a proportion exits through the

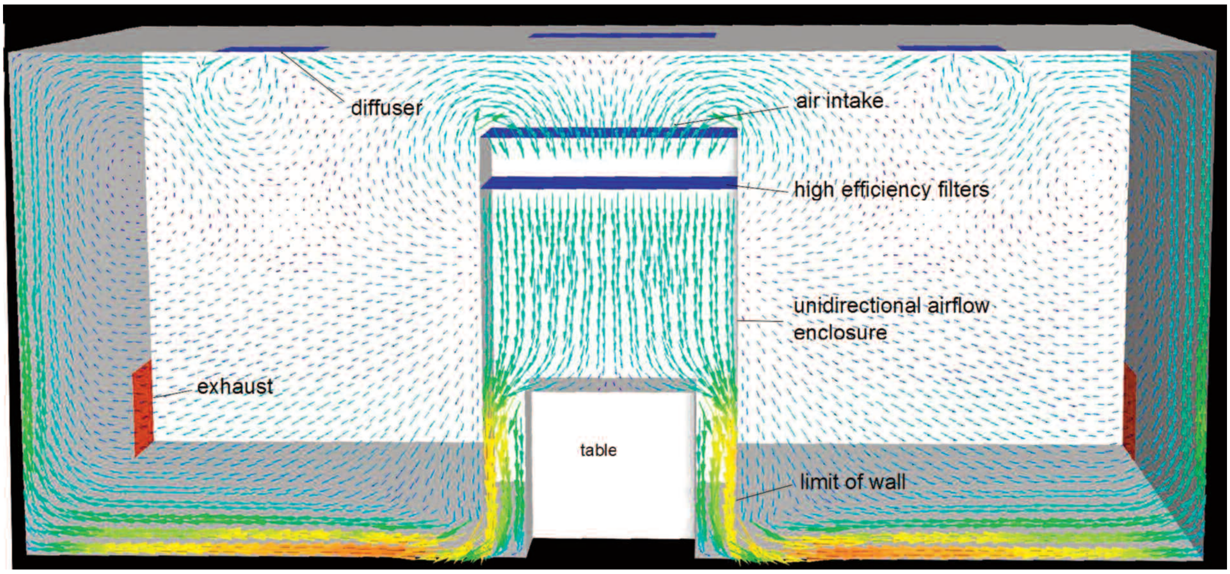

Figure 3. Airflow in a non-UDAF cleanroom containing a free-standing vertical-UDAF workstation.

cleanroom's low-level extracts without effectively mixing with room air. Article IV discusses the proportion of air (β) from clean air devices that effectively mixes with room air, with about 0.5 in the situation shown in **Figure 3**, about 0.2 when the same type of workstation is supplied from the air handling plant that also supplies the cleanroom, and about 0.8 from small clean air devices. If the amount of air passing through the device is Q_D and the ventilation effectiveness coefficient is β, then the air supply from the air handling plant to the cleanroom can be reduced by βQ_D. It should be noted that the presence of a clean air device is likely to influence the ventilation effectiveness index, and may give an index different from that reported in Article III for non-UDAF cleanrooms with no clean air device. This situation is discussed in the *Worked examples of the calculation of air supply rates or airborne concentrations in a non-UDAF cleanroom* section.

Surface deposition of particles and MCPs

The air supply rate required for a non-UDAF cleanroom can be calculated by Equation 4, and the airborne concentration of particles and MCPs by Equation 5. Both equations incorporate the effect of surface deposition and, to obtain this, the deposition velocity is required. Deposition velocities of a range of cumulative particle sizes have been reported in Article VI, where it is also reported that the deposition velocity may increase as the airborne concentration decreases. Small particles ≥0.3 µm and ≥0.5 µm were shown to have deposition velocities of 2.8 x 10^{-5} m/s and 6.4 x 10^{-5} m/s, repectively, and the surface deposition over a range of air cleanliness is very low, and it is unnecessary to include their deposition in these calculations. However, particles ≥5 µm have greater deposition rates, and their deposition should be included.

In Article VI, Hamberg's research in cleanrooms is discussed[19], which shows that for particles ≥5 µm, the particle deposition rate (PDR) is related to the airborne concentration (C) in different cleanliness conditions by the following equation (converted to SI units).

$$\text{PDR}_{\geq 5\mu\text{m}}\,(\text{no./m}^2/\text{s}) = 0.0226 \times \text{C}^{0.773}$$

However, it is known that

$$V_D = \frac{PDR}{C}$$

Therefore,

Equation 12

$$V_D(m/s) = \frac{0.0226 \times C^{0.773}}{C}$$

Because of the difficulty in accurately measuring low airborne concentrations of particles ≥5 µm, the class limits for that size of particle are only specified in Classes 6, 7, 8 and 9 of ISO 14644-1 (2015)[1]. The deposition velocities for particles ≥5 µm, calculated by means of Equation 12, are given in **Table 4** for these classes.

It has been additionally reported in Article V that the deposition velocities of MCPs are related to the airborne concentration, and the microbial deposition rate (MDR) given by the following equation.

$$\text{MDR (no./m}^2\text{/s)} = 0.0161 \times C^{0.6571}$$

Using the same approach as described above for particles ≥5 µm, the deposition velocities expected in the different Grades of cleanrooms specified in Annex 1 of EU GGMP[2] are given in **Table 5**. Additonal depositon velocities for other airborne concentrations are reported in Article V.

Table 4. Deposition velocities of particles ≥5 μm in ISO cleanliness classes.		
ISO 14644-1 Class[1]	**Class limit for particles ≥5 μm/m³**	**Deposition velocity (m/s)**
6	293	0.0062
7	2930	0.0037
8	29,300	0.0022
9	293,000	0.0013

Calculation of the increase of air supply rate to ensure the airborne concentration limit is rarely exceeded

The dispersion rates of particles and MCPs from sources will vary in a cleanroom over time and are normally reported as an average number per second and, therefore, the calculated air supply rate gives an average airborne concentration (number/m³), where half the counts will be above the average concentration. However, ISO 14644-1[1] requires the particle concentration not to exceed the class limit, and a cleanroom with an airborne concentration whose limit is exceeded about half the time, would be generally unacceptable. It is almost impossible to design a cleanroom that will never exceed the class limit, as airborne counts conform to a statistical distribution, with counts distributed around the average and a number of outlying high counts. By increasing the air volume supply rate, the percentage of airborne counts above the class limit will decrease, and the increase in air supply to ensure that an ISO class limit is rarely exceeded can be calculated using the method explained in Article IV. Given in **Table 6** are the number of times the air supply rate should be increased to ensure that 95% or 99% of the counts are below the class limit.

The increases of air supply given in **Table 6** are based on the coefficient of variation (C_V) and the percentage of particle counts required to be below the class limit. The coefficient of variation shows how widely spread airborne counts are from the average, and is the ratio of standard deviation to the mean of the counts. Values of C_V found in cleanrooms are given in Article IV and are normally between 0.5 and 2, and a value of 1 is a reasonable choice. The percentage of counts below the class limits can be chosen as 95% as this is a reasonable choice for two reasons. Firstly, ISO 14644-1 (2015) selects the number of sampling locations to give a 95% chance that 90% of the count will be below the class limit. Secondly, ISO 14644-1 (2015) allows a second chance of sampling a location when a count is over the class limit, although this is only allowed when there is an abnormal occurrence. However, in most situations there is enough uncertainty to accept the likelihood of an abnormal occurrence. As 95% is a proportion of 0.05, the chance that two consecutive counts will be above the limit is 0.05 x 0.05 = 2.5 in 1000. Although 95% seems a reasonable level, there may be situations where higher percentages of counts (and values of C_V) may be desirable, and the increases in air supply required in such conditions are available in Article IV.

Effect of different types of air supply distribution systems

Equations 1 to 5 have been derived to calculate the airborne concentration of contamination, or the air supply rate needed for a specified concentration of contamination. However, there are variables not included

Table 5. Deposition velocities for different airborne concentrations of MCPs.		
EU GGMP Annex 1[2] cleanroom grades	**Airborne MCP concentration/m³**	**Deposition velocity (cm/s)**
A	1	0.016
B	10	0.0073
C	100	0.0033

Table 6. Required increase in air supply rates.		
Percentage of counts below maximum concentration	**Ratio of standard deviation to mean (C_V)**	**Number of times increase in air supply rate**
95%	1	2.7
99%	1	3.5

in these equations that might affect the results, namely,

(a) the design of the air conditioning plant with respect to the mixing of recirculated and fresh air, and the number, placement and combinations of air filters;
(b) removal efficiency of the air filters;
(c) concentration of contamination in the outside air;
(d) percentage of fresh make-up air in total air supply.

To investigate the significance of these additional variables, equations were derived in Article VII that included these variables, in addition to the already established variables of airborne dispersion rate and surface deposition. Equations were derived for the steady-state condition in three common layouts of air handling plants, when an additional secondary air filter was installed, or not. The importance of all equation variables was then investigated in Article IX.

In Article IX, the concentration of particles ≥0.5 µm and MCPs in the air of a typical non-UDAF cleanroom was calculated for various designs of ventilation plants, using different placements and combinations of air filters. Filter standards EN 1822-1:2009[20] and ISO 29463-1:2009[21] classify high efficiency filters by their removal efficiency against the most penetrating particle size (MPPS). It was found that a primary/secondary/terminal combination of air filters with removal efficiencies of 85/99.95/99.995% (a E10/H13/H14 filter combination according to EN 1822), provided no contribution of particles ≥0.5 µm in the supply air. If a secondary filter is not installed, a few particles were supplied to the cleanroom. This filter combination appeared to be satisfactory, especially as the post-installation leak test to demonstrate that terminal filters are free of leaks, requires the penetration of test particles to be less than 0.01%. To ensure this, the filter's local penetration should be close to 99.99%, i.e. the terminal filter is not less efficient than a H14 filter. However, should other combinations of filters be considered, the equations derived in Article VII will establish their effectiveness in removing particles and MCPs from the air supply.

If the installed air filters ensure that the contamination in the supply air is practically zero, the most important determinants of airborne concentration in a cleanroom were demonstrated in Article IX to be the air supply rate and dispersion rate of contamination, with a lesser effect from surface deposition. The additional variables listed at the start of this section were shown to be of little practical importance when effective air filters, typical of the type installed in current designs of cleanrooms, are installed. The previously quoted Equations 4 and 5 were, therefore, shown to be suitable equations for the calculation of the air supply rate, or airborne concentration in non-UDAF cleanrooms.

Ensuring air supply rates comply with EU GGMP (2008)[2] and ISO 14644-3: 2005[1] recovery rate requirements

Pharmaceutical cleanrooms should meet the 'clean up' requirements given in Annex 1 of EU GGMP (2008)[2], and other cleanrooms may be required to meet a recovery rate requirement according to ISO 14644-3: 2005[1]. Both 'clean up' and 'recovery rate' tests measure the particle decay rate which, as previously discussed, is dependent on the cleanroom's air change rate.

Article VIII describes a method to calculate the air change rates needed to achieve the 'clean up' requirements of the EU GGMP. The method takes account of the ventilation effectiveness of the airflow in the cleanroom. If the ACE index is assumed to be no poorer than 0.7, then, if the most stringent requirements are applied to Grade B cleanrooms of a 100-fold drop in particle concentration from the 'in operation' to 'at rest' state, in 15 minutes, the required air changes per hour is 26. For Grade C cleanrooms that require a 10-fold drop in 15 minutes, 13 air changes per hour are required. Other air change rates can be calculated for different conditions.

Knowing the volume of the cleanroom, the air supply rate calculated by the method described in the previous sections should be converted to the room's air change rate. This air change rate should be the same as, or greater than, the air change required for the EU GGMP 'clean up' requirements.

The same calculation method used for the EU GGMP 'clean up' requirement can also be used to check that the air supply rate (converted to an air change rate) will achieve the required recovery rate according to ISO 14644-3[11], for any drop in particle concentration, time requirement, or ventilation effectiveness index.

Worked examples of the calculation of air supply rates or airborne concentrations in a non-UDAF cleanroom

Calculation of the air supply rate

It is assumed that air supply rates required to satisfy the temperature, humidity, and other ventilation requirements of a cleanroom, will be separately calculated. This section only considers the air supply rate to provide a specified airborne concentration of particle and MCP contamination. The method is demonstrated by a worked example of a non-UDAF cleanroom that has to conform to a cleanliness standard of ISO 14644-1 Class 7 at particle sizes of ≥0.5 µm (352,000/m^3) and ≥5 µm (2930/m^3), and an MCP limit of 10/m^3. It also has to comply with the 'clean-up' requirement of the EU GGMP (2008) for a Grade B cleanroom.

The combination of filters to be installed are chosen to ensure that the air supply contributes little, or no, airborne contamination to the cleanroom and, to achieve this, reference should be made to the *Effect of different types of air supply distribution systems* section and Articles VII and IX. When the number, placement and removal efficiency of the filters has been established, the air supply rate can be calculated by means of Equation 4.

$$Q = \frac{D}{\varepsilon C} - \beta Q_D - V_D A$$

When there are no clean air devices, the second term can be removed from the equation, and if surface deposition is not included, the third term can be removed. In this example, deposition is included, and the calculation is firstly carried out with no clean air device present, and when one is installed.

Step 1 in calculating the air supply rate is to obtain the dispersion rate of airborne contamination in the cleanroom. In this example, dispersion comes from two people working in the cleanroom, and machinery. If the new cleanroom will have the same production method as an existing cleanroom, then the dispersion rate can be obtained from the existing room by the method described in the *Dispersion rates from personnel and machinery* section. However, the cleanroom was required for a new production method, and the dispersion rate can be obtained by simulating the new production method in a cleanroom, and using the method given in the *Dispersion rates from personnel and machinery* section. If no experimental information on dispersion rates is available, then it can be estimated from data similar to that given in the *Dispersion rates from personnel and machinery* section. The two people working in the cleanroom will wear one-piece polyester coveralls with hood and overboots, and will be active most of the time. It was decided that the average dispersion rates in **Table 1** would be used, and for two people it was 34,000/s for particles ≥0.5 μm, 1200/s for particles ≥5 μm, and 6/s for MCPs. The manufacturer reported that the machinery dispersed 500/s of particles ≥0.5 μm, and 100/s of particles ≥5 μm, but no MCPs. The total sum of the dispersion rates (D) is given in **Table 7**.

Step 2 establishes the value of the ventilation effectiveness index (ε), and reference should be made to the *Ventilation effectiveness* section for further information on this topic. It was decided to avoid uneven concentrations of contamination around the cleanroom in order to minimise the need for extra air to compensate for the higher concentrations of contamination that might occur. Therefore, effective air diffusers were used to supply and mix the filtered air, with low-level extracts around the periphery of the cleanroom. This should give a ventilation effectiveness index close to 1 but, to add a safety margin, a value of 0.7 was assumed.

Step 3 establishes the floor area (A) and particle deposition velocities (D_V) that are needed to calculate the reduction of the airborne concentration owing to surface deposition losses. The total horizontal surface area of the cleanroom is assumed to be the same as the floor area of 50 m^2. The deposition velocities of particles ≥5 μm and MCPs are given in **Tables 4** and **5**. The deposition velocity of particles ≥0.5 μm was not included, as the deposition was known to be insignificant (see the *Surface deposition of particles and MCPs* section).

Step 4 calculates the initial value of the air supply rate. It was firstly assumed that no clean air device was installed, and Equation 4 without the clean air device term was used to calculate the air supply rate.

Table 7. Calculation of the air supply rates.

Airborne contamination type	≥0.5 μm	≥5 μm	MCPs
Maximum concentration of airborne contamination /m^3 – C	352,000	2930	10
Total dispersion rate/s – D	34,500	1300	6
Ventilation effectiveness index – ε	0.7	0.7	0.7
Deposition velocity (m/s) – V_D	–	0.0037	0.0073
Floor area (m^2) – A	50	50	50
Without clean air device			
Calculated air supply from air handling plant (m^3/s) including surface deposition	0.14	0.45	0.49
Air supply rate uplifted so maximum concentration is rarely exceeded (x 2.7)	**0.37**	**1.21**	**1.33**
Room volume (m^3)	160	160	160
Air change rate/hour	8	27	30
With addition of clean air device			
Air volume supply of clean air device (m^3/s) – Q_D	3.6	3.6	3.6
Ventilation effectiveness coefficient of device – β	0.2	0.2	0.2
Air supply to cleanroom from device – βQ_D	0.72	0.72	0.72
Calculated air supply rate (m^3/s) from heating, ventilation and air-conditioning (HVAC) plant using the uplifted supply rate and deduction owing to surface deposition and device – Equation 3	–0.35	0.49	0.61

$$Q = \frac{D}{\varepsilon C} - V_D A$$

The results of this calculation are given in **Table 7**, where it can be seen that the highest air supply rate required to achieve the specified concentrations for all three contaminants is that calculated for MCPs. It is 0.49 m^3/s, with a slightly lower supply rate required for particles ≥5 µm (0.45 m^3/s), and the lowest rate for particles ≥0.5 µm (0.14 m^3/s). However, had cleanroom clothing been more effective in reducing the dispersion rate of MCPs than for particles, or machinery found to emit larger quantities of smaller particles, the air supply rate might have to be based on the requirement of particles ≥0.5 µm, or ≥5 µm.

Step 5 determines the uplift in the air supply to ensure that airborne counts in the cleanroom will rarely exceed the ISO class limits. The chosen percentage of airborne counts below the class limit is set at 95%, with a C_V of 1. For this requirement, **Table 6** shows that the air supply rate has to be uplifted 2.7 times. The uplifted air supply rates for each of the three contaminants are given in **Table 7**, and it can be seen that the highest rate is 1.33 m^3/s. This air supply rate should now be compared with the other rates calculated to satisfy temperature, humidity, and other ventilation requirements, and the highest rate chosen. In this example, it is assumed that it is the one required for contamination control, and is 1.33 m^3/s.

Step 6 is only carried out if the cleanroom has to comply with the 'clean up' requirements of the EU GGMP, or a recovery rate specified according to ISO 14644-3. In this example, the cleanroom has to comply with the EU GGMP 'clean up' requirement for a Grade B cleanroom. The ACE index is assumed to be 0.7, and, therefore, according to the *Calculation of the increase of air supply rate to ensure the airborne concentration limit is rarely exceeded* section, the air change rate/hour should not be less than 26. Knowing the room volume is 160 m^3, the air supply rate of 1.33 m^3/s is converted to an air change rate, which is 30 air changes per hour. This air change rate is, therefore, more than sufficient to ensure that the EU GGMP 'clean-up' requirement will be achieved. Had the air change rate been below 26 air changes per hour, the air supply rate would have to be increased.

The result of the above calculation of a required air supply rate of 1.33 m^3/s did not consider the contribution of a clean air device, and this is now calculated by means of Equation 4 that includes the clean air device term.

$$Q = \frac{D}{\varepsilon C} - \beta Q_D - V_D A$$

An example is taken of a cleanroom where a UDAF workstation is installed that has a filter face area of 3 m x 3 m and filter face velocity of 0.4 m/s. Therefore, the air volume rate passing through the device (Q_D) is 3.6 m^3/s. The air supplied to the device came from the air handling plant and the ventilation effectiveness coefficient (β) is assumed to be 0.2 and, therefore, the additional clean air contribution from the device (βQ_D) is 0.72 m^3/s. The air supply rate to the cleanroom from the air handling plant can be reduced by this amount and the recalculated air supply rates for the three contaminants are given in **Table 7**. The highest air supply rate from the air handling plant that is needed to control all three types of contaminants is 0.61 m^3/s. This rate should be compared with those calculated for control of temperature, humidity, or other requirements, and the highest value adopted. The value of 0.61 m^3/s was the highest value, and is the final air supply rate required for the cleanroom.

It should be noted that airflow from a clean air device is likely to cause a change to the airflow pattern in the cleanroom. This may result in a ventilation efficiency index (ε) that is different from that expected in a cleanroom where a device is not installed. The value of β may also be unclear. To clarify this situation, a CFD analysis can be carried out in which the effect of the clean air device (β) and the ventilation effectiveness (ε) can be determined as one variable (α), and the following modified equation used.

$$Q = \frac{D}{\alpha C} - V_D A$$

In the present calculation, a simple non-CFD approach has been used with Equation 4.

It can be seen in **Table 7** that the air supply rate required for particles ≥0.5 µm, when the contribution from the clean air device is included, is negative. This shows that the clean air device provides more air than required to control particles ≥0.5 µm. However, there must be sufficient supply air to pressurise the cleanroom and control temperature and humidity, and the highest air supply requirement should be chosen.

When a clean air device is installed, the air supply rate from the air handling plant is reduced but the total 'effective' air supply is the same as when the clean air device is not present. Therefore, the air change rate of the cleanroom with clean air device to achieve the 'clean-up' requirement of the EU GGMP or ISO 14644-3 is the same as previously calculated.

Calculation of airborne concentration

The calculation method of the airborne concentration of contaminants in a non-UDAF cleanroom is demonstrated by a cleanroom with similar properties to that in the previous section, and these are shown in **Table 8**. To simplify the calculation, the clean air device is not included, but the effect of surface deposition of particles ≥5 µm and MCPs is included, but not particles ≥0.5 µm. Equation 5 with the clean air device term excluded is as follows.

$$C = \frac{D}{\varepsilon(Q + V_D A)}$$

The air supply rate is assumed to be 0.49 m3/s, which was the rate calculated in the previous section for a cleanroom

Table 8. Calculation of airborne contaminants in a non-UDAF cleanroom without clean air device.

Airborne contamination type	≥0.5 *μm*	≥5 *μm*	MCPs
Dispersion rate/s – ***D***	34,500	1300	6
Air supply from HVAC plant to non-UDAF cleanroom (m^3/s) – ***Q***	0.49	0.49	0.49
VE index – ***ε***	0.7	0.7	0.7
Deposition velocity (m/s) – D_V	–	0.0037	0.0073
Floor area (m^2) – ***A***	50	50	50
Calculated airborne concentration/m^3 – C	**99,930**	**2751**	**10**

without a clean air device, and based on the highest air supply rate needed to ensure that MCPs (and hence the two other types of contamination) achieve the specified airborne concentration. This air supply rate does not include an uplift of the air supply. The results of the calculation of the expected airborne concentrations of the three contaminants are given in **Table 8**.

Discussion

This article reviews scientific articles published by the authors to provide methods for calculating in non-UDAF cleanrooms, either the airborne concentrations of particles and MCPs, or the air supply rate required to achieve specified concentrations of airborne contamination. These are calculated for the steady-state condition, which is the condition where the contamination of product or production process may occur. In that condition, the contribution of various combinations of air filters were investigated, and it was shown that air filters of the type routinely installed in cleanrooms ensure that there is little, or no, contribution to the airborne contamination from the air supply (assuming no leakage from the filter system) to non-UDAF cleanrooms. In this situation, the airborne concentration in a non-UDAF cleanroom, or the air supply rate, can be calculated by Equations 1 to 5 using combinations of air supply rate, concentration of airborne contamination, dispersion rate of contamination, ventilation effectiveness index and, when installed, the contribution of clean air devices. Should lower removal efficiency air filters be utilised, or other design decisions made that might allow particles to enter in the supply air, then Articles VII and IX give equations that can be used to calculate the outcome of these design decisions. The effect of particle deposition should be included in the calculations involving MCPs and particles ≥5 µm, but can be ignored for particles ≥0.3 µm and ≥0.5 µm. Worked examples are provided to demonstrate calculations of air supply rate and particle concentration.

Also discussed in this article is the decay of particles in a cleanroom, which is determined by the room's air change rate. Extending the ISO 14644-3 recovery rate test method allows an ACE ventilation effectiveness index to be obtained that gives a numerical result of the cleanroom's effectiveness of removing airborne contamination. The EU GGMP 'clean up' test was also considered, and a method devised for calculating the air change rate needed to achieve the desired 'clean-up' requirements.

To calculate the air supply rate, or concentrations of airborne contamination, it is necessary to obtain values of the equation variables, and these are provided in this article and in Article IV. If this information is accurate then so will the results, but there may be uncertainty in some of the values. However, the calculation method includes a requirement to increase the air supply rate by several-fold to ensure that counts rarely exceed the class limit and this provides a margin of safety.

The success of these calculations has been assessed in Article IV by comparing airborne concentrations with the concentration calculated from the actual air supply in a non-UDAF cleanroom, as well as similar unpublished observations carried out by authors of this article, and good correlation has been found. When compared to the method commonly used at present to design a non-UDAF cleanroom, which is mainly based on experience, the analytical methods in this article provide a useful step forward.

Main articles

I. Whyte W, Whyte WM and Eaton T. The application of the ventilation equations to cleanrooms Part 1: The equations. *Clean Air and Containment Review* 2012;Issue **12**:4–8. Available at: http://eprints.gla.ac.uk/84186/1/84186.pdf

II. Whyte W, Whyte WM and Eaton T. The application of the ventilation equations to cleanrooms Part 2: Decay of contamination. *Clean Air and Containment Review* 2014;Issue **20**:4–9. Available at: http://eprints.gla.ac.uk/100820/1/100820.pdf

III. Whyte W, Ward S, Whyte WM and Eaton T. Decay of airborne contamination and ventilation effectiveness of cleanrooms. *International Journal of Ventilation* 2014;**13**(3):211–219. Available at: http://eprints.gla.ac.uk/100819/1/100819i.pdf

IV. Whyte W, Whyte WM, Eaton T and Lenegan N. Calculation of air supply rates for non-unidirectional airflow cleanrooms. *European Journal of Parenteral and Pharmaceutical Sciences* 2014;**19**(4):121–129. Available at: http://eprints.gla.ac.uk/104331/1/104331.pdf

V. Whyte W and Eaton T. Deposition velocities of airborne microbe-carrying particles. *European Journal of Parenteral and Pharmaceutical Sciences* 2016;**21**(2):45–49. Available at: http://eprints.gla.ac.uk/121686/1/121686.pdf

VI. Whyte W, Agricola K and Derks M. Airborne particle deposition in cleanrooms: relationship between deposition rate and airborne concentration. *Clean Air and Containment Review* 2016;Issue **25**:4–10. Available at: http://eprints.gla.ac.uk/119091/1/119091.pdf

VII. Whyte W, Lenegan N and Eaton T. Equations for predicting airborne cleanliness in non-unidirectional airflow cleanrooms. *European Journal of Parenteral and Pharmaceutical Sciences* 2016;**21**(2):38–43. Available at: http://eprints.gla.ac.uk/121682/1/121682.pdf

VIII. Whyte W, Lenegan N and Eaton T. Ensuring the air supply rate to a cleanroom complies with the EU GGMP and ISO 14644-3 recovery rate requirements. *Clean Air and Containment Review* 2016;Issue **26**:22–24. Available at: http://eprints.gla.ac.uk/119087/1/119087.pdf

IX. Whyte W, Lenegan N and Eaton T. Calculation of airborne cleanliness and air supply rate for non-unidirectional airflow cleanrooms. *European Journal of Parenteral and Pharmaceutical Sciences* 2016;**21**(3):79–88. Available at: http://eprints.gla.ac.uk/130348/1/130348.pdf

Additional references

1. International Organization for Standardization. *ISO 14644-1. Cleanrooms and Associated Controlled Environments. Part 1: Classification of Air Cleanliness by Particle Concentration*. Geneva, Switzerland: ISO; 2015.
2. European Commission. *EudraLex. The Rules Governing Medicinal Products in the European Union. Volume 4: EU Guidelines to Good Manufacturing Practice – Medicinal Products for Human and Veterinary Use. Annex 1 – Manufacture of Sterile Medicinal Products*. Brussels, Belgium: European Commission; 2008.
3. Food and Drug Administration. *Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice*. Silver Spring, MD, USA: FDA; 2004
4. Morrison PW. *Environmental Control in Electronic Manufacturing*. New York, NY, USA: Van Nostrand; 1973. ISBN: 0442255640.
5. Brown WK and Lynn CA. Fundamental clean room concepts. *ASHRAE Transactions* 1986;**92**(1):272–288.
6. Gustavsson J. Calculating cleanroom cleanliness. *Cleanroom Technology* 1999;**5**(1):24–25.
7. Jaisinghani RA. Energy efficient low operating cost cleanroom airflow design. Presentation at Institute of Environmental Science and Technology Estech Conference, Phoenix, AZ, USA; 2003.
8. Sun W, Mitchell J, Flyzik K, Shih-Cheng H, Junjie L, Vijayakumar R and Fukuda H. Development of cleanroom required airflow rate model based on establishment of theoretical basis and lab validation. *ASHRAE Transactions* 2010;**116**:87–97.
9. Camfil Farr. *Clean Room Design Standards and Energy Optimisation*. Stockholm, Sweden: Camfil Farr; 2012.
10. Fedotov A. Air change rate for cleanrooms with non-unidirectional airflow. *Clean Air and Containment Review* 2016;Issue **26**:12–20.
11. International Organization for Standardization. *ISO 14644-3. Cleanrooms and Associated Controlled Environments. Part 3: Test Methods*. Geneva, Switzerland: ISO; 2005.
12. American National Standards Institute. *ANSI/ASHRAE Standard 129-1997 (RA 2002). Measuring Air-Change Effectiveness*. Washington, DC, USA: ANSI; 1997.
13. International Organization for Standardization. *ISO 14644-14. Assessment of Suitability of Equipment and Materials for Cleanrooms*. Geneva, Switzerland: ISO; 2017.
14. Institute of Environmental Science and Technology. *IEST-RP-CC003: Garment System Considerations for Cleanrooms and Other Controlled Environments*. Schaumburg, IL, USA: IEST; 2011.
15. Hejab M. Prediction of airborne contamination in conventionally ventilated cleanrooms. PhD Thesis, University of Glasgow, Scotland; 1992.
16. Sundstrom S, Ljungqvist B and Reinmuller B. Some observations on airborne particles in blow-fill-seal filling rooms. *PDA Journal of Pharmaceutical Science and Technology* 2007;**61**(3):147–153.
17. Hnatek ER. *Integrated Circuit Quality and Reliability*, Second edition. Boca Raton, FL, USA: CRC Press; 1995.
18. Mundt E (Ed.), Mathisen HM, Nielsen PV and Moser A. *Guidebook No. 2 – Ventilation Effectiveness*. Brussels, Belgium: REHVA (Federation of European Heating and Ventilation and Air-Conditioning Association); 2004.
19. Hamberg O. Particle fallout predictions for clean rooms. *The Journal of Environmental Sciences* 1982;**25**:15–20.
20. European Committee for Standardization. *EN 1822-1. High Efficiency Air Filters (EPA, HEPA and ULPA). Classification, Performance, Testing, Marking*. Brussels, Belgium: European Committee for Standardization; 2009.
21. International Organization for Standardization. *ISO 29463-1. High-Efficiency Filters and Filter Media for Removing Particles in Air. Part 1: Classification, Performance Testing and Marking*. Geneva, Switzerland: ISO; 2011.