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# Microbial risk assessment in pharmaceutical cleanrooms

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**The microbial risk to aseptically manufactured products in pharmaceutical cleanrooms can be assessed by the use of fundamental equations that model the dispersion, transfer and deposition of microbial contamination, and the use of numerical values or risk descriptors. This can be done in two-stages, with the first stage used to assess the transfer of contamination from all of the sources within the cleanroom suite and the second stage used to assess both air and surface contact contamination within critical production areas. These two methods can be used to assess and reduce microbial risk at the preliminary design stage of the cleanroom and associated manufacturing process or, retrospectively, for an established manufacturing operation.**

## Introduction

A number of systems exist for managing risk during manufacturing. These include Fault Tree Analysis (FTA)<sup>1</sup>, Failure Mode and Effect Analysis (FMEA)<sup>2</sup>, Hazard and Operational Studies (HAZOP)<sup>3</sup>, and Hazard Analysis and Critical Control Point (HACCP)<sup>4</sup>. The HACCP system, having been developed for the food industry, appears to be the most suitable risk management system for the methodical assessment, control and monitoring of microbial risk in the pharmaceutical industry<sup>5</sup>.

An important part of a risk management system is the risk assessment process, where the importance or degree of risk associated with each identified hazard is assessed. Each hazard is assigned a numerical value or descriptor of risk. Those hazards with a high degree of risk can then be considered further in order to reduce the risk to a more acceptable level. A useful risk assessment method is the FMECA criticality method outlined in International Electronic Commission report on the procedure for Failure Mode and Effect Analysis (FMEA)<sup>2</sup>. The FMECA method is based on the following equation:

### Equation 1

Risk = criticality of the occurrence × frequency of occurrence

This risk equation was considered in a previously published paper<sup>6</sup>, and shown to be more correct than a similar equation that includes a third variable of ‘detection’. It was also established that ‘criticality’, when considering microbial contamination, reflects the importance of a hazard in terms of the number of microbes from the hazard i.e. source, that are deposited onto the product; this should be expressed in terms of the concentration of microbes on, or in, a source, and their

likelihood of dispersion, transfer, and deposition. The ‘frequency’ variable should be expressed as either the time the product is exposed, or the frequency of contamination incidents. If this is accepted, then the criticality risk assessment **Equation 1** is the same as the fundamental equations described below, and its scientific basis vindicated.

To accurately assess microbial risk, the basic models of risk must be known so that the fundamental variables that predict risk and their importance in relation to the other variables can be determined. The overall equation that applies to the risk of microbial contamination of a product has been derived<sup>6</sup> and is as follows:

### Equation 2

No. of microbes deposited on a product =  $C \times S \times P_d \times P_a \times A \times T$

where:

- C = concentration of microbial contamination on, or in, a source (number/cm<sup>2</sup> for a surface, or number/cm<sup>3</sup> for air);
- S = the quantity of surface material, or air, that is dispersed, from a source in a given time (cm<sup>2</sup> /s for surfaces, and cm<sup>3</sup> /s for air dispersion); this can also be expressed as the quantity dispersed per frequency of occurrence;
- P<sub>d</sub> = proportion of microorganisms dispersed from a source that are transferred to the area adjacent to the product;
- P<sub>a</sub> = proportion of microorganisms in the adjacent area that are deposited per unit area of the product (/cm<sup>2</sup>);
- A = area of surface onto which microbes are deposit (cm<sup>2</sup>);
- T = time, during which transfers occur(s); this can also be expressed as frequency of occurrence.

Two simplified versions of **Equation 2** were derived<sup>6</sup> to model surface contact and airborne deposition of microbes into, or onto, a product. **Equation 3** models surface contact contamination:

### Equation 3

Number of microorganisms deposited by surface contact over

a given time (no.) = microbes on contaminating surface (no./cm<sup>2</sup>) x transfer coefficient x area of product that is contacted (cm<sup>2</sup>) x frequency of contact

where:

the 'transfer coefficient' is the proportion of microorganisms on a contaminating surface (such as a glove) that are transferred to the product.

**Equation 4** models airborne deposition onto a product and is as follows:

**Equation 4**

Number of airborne microorganisms deposited onto the product in a given time (no.) = deposition rate of microbe carrying particles (no./(cm<sup>2</sup>.s)) x area of product exposed (cm<sup>2</sup>) x time of exposure (s)

Most microbes in the cleanroom air are rafted on particles of skin, their average size being between about 8µm and 20µm and these microbe carrying particles will deposit into the product, mainly by gravity<sup>7</sup>. The deposition rate of microbe carrying particles into, or onto, a product can be ascertained from settle plates exposed adjacent to the exposed product<sup>8</sup>.

To obtain the most accurate estimate of the amount of microbial contamination by airborne deposition or surface contact, numerical values should be substituted into the above equations. However, in pharmaceutical production, much of this numerical information is unknown and it is therefore necessary to employ risk assessment methods using descriptors that act as surrogates for the required numerical data. To obtain the highest degree of accuracy from the use of descriptors, those that best describe the variables in the above equations should be chosen. They should be combined as indicated in the equations, so that the relative importance of the variables is retained. This can be done using the FMECA method as described in the following sections of this paper.

**Overall deposition model**

It is useful to start a microbial risk assessment of a pharmaceutical production area by considering all of the microbial hazards in the various clean areas in the manufacturing suite; a detailed consideration of critical areas will be carried out later. In this paper, all microbial sources are considered to be potential hazards. To carry out an overall risk assessment, the analysis should be based on **Equation 2**. The concentration of microbes in the air, or on surfaces, is one of the required variables and numerical values should be available, and used. However, numerical values are likely to be unavailable for other variables in the equation. The time duration, or frequency of microbial contamination in cleanroom, can be continuous or unknown. For example, the rate of

deposition of microbes from cleanroom air is continuous, the rate not varying significantly during production. Also, the frequency of incidents of surface contamination, such as when personnel touch the product, is normally unknown, personnel often being unaware of its occurrence. Thus, for the purpose of an overall risk assessment, the variable of time (T) is not used. Numerical information is also rarely available about the dispersion, transfer and deposition of micro-organisms. Consequently, a simplified version of the equation using surrogate descriptors for these unattainable variables is shown as **Equation 5** and should be used for the analysis.

**Equation 5**

Risk from microbial contamination (risk rating) = A x B x C x D

where:

- A = microbial contamination on, or in, a source;
- B = ease of dispersion and transfer;
- C = proximity of source from critical area;
- D = effectiveness of control method.

The risk rating of each source is determined by assigning risk scores to risk factors A to D. Given in **Table 1** is an example of risk scores that can be used. It should be noted that the chance of transfer of contamination (risk factor C) is assessed from the distance the source is from the product. This is a reasonable approach, but may need modification in some circumstances e.g. a person upstream of the product in a unidirectional air flow will have a different chance of contaminating the product than someone standing the same distance downstream. Other risk factors may need similar modification.

A comprehensive identification of all the sources of microbial risk, is fundamental to the success of this approach. A method to identify and group sources of microbial contamination has been discussed by Whyte<sup>5</sup>. Shown in **Figure 1**, in Mind Map format, are the groupings of microbial sources that should be considered. During an actual assessment, a more extensive identification of sources is expected. Also shown in **Figure 1** are the connections between people and other sources, emphasising the fact that people are the primary source of microbes in a cleanroom.

| <b>Table 1: Scores for risk factors used for assessing hazards</b> |   |  |  |
|--|---|--|--|
| <b>Risk factor (A)</b>   | <b>Risk factor (B)</b>                                    | <b>Risk factor (C)</b>                                   | <b>Risk factor (D)</b>                 |
| <b>Amount of microbial contamination on, or in, a source</b>       | <b>Ease of dispersion, or transfer, of microorganisms</b> | <b>Proximity (location) of source from critical area</b> | <b>Effectiveness of control method</b> |
| 0 = nil  | 0 = nil   | 0 = remote   | 0 = full barrier control*              |
| 0.5 = very low   | 0.5 = very low  | 0.5 = in outside corridor, air lock                      | 0.5 = very good control                |
| 1 = low  | 1 = low   | 1 = periphery of cleanroom                               | 1 = good control                       |
| 1.5 = medium   | 1.5 = medium  | 1.5 = general area of cleanroom                          | 1.5 = some control                     |
| 2 = high   | 2 = high  | 2 = critical area  | 2 = no control                         |
| *Complete physical barrier between source and critical area        |   |  |  |

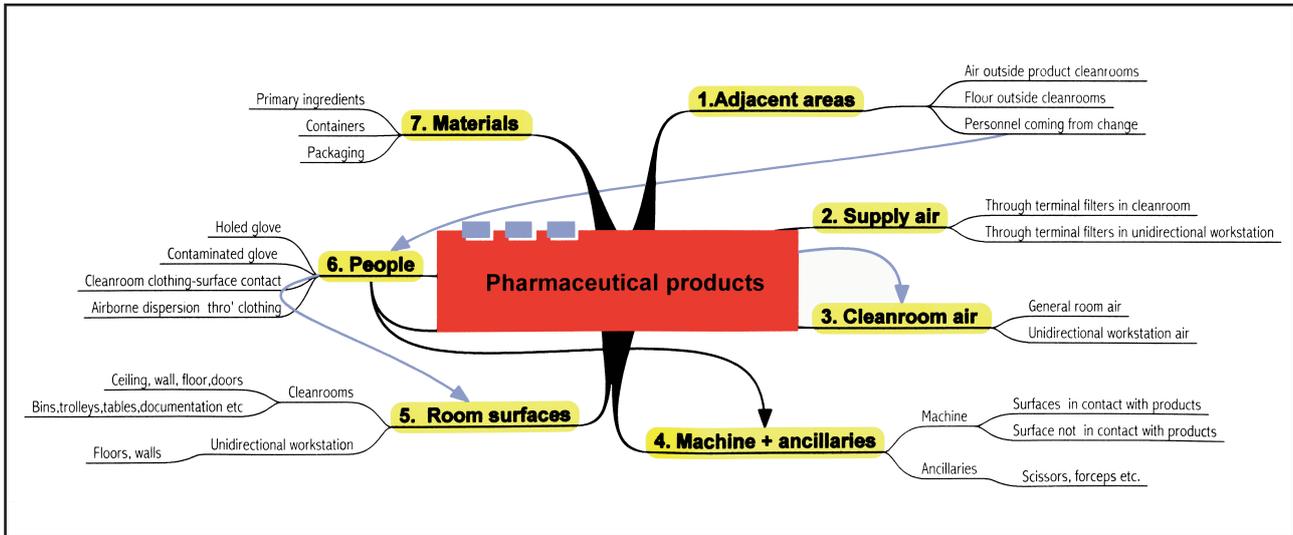


Figure 1. Microbial sources in a cleanroom.

**Table 2.** Calculation of the risk rating for an identified source.

| Group and Source                                 | Risk factor A.<br>Conc. of microbes | Risk factor B.<br>Ease of movement to product | Risk factor C.<br>Proximity to product | Risk factor D.<br>Effectiveness of control (method given)             | Risk rating |
|--|-------------------------------------|---|--|---|-------------|
| <b>1. Areas adjacent to production cleanroom</b> |                                     |   |  |   |             |
| 1.a Air outside production cleanroom (corridor)  | 1.5                                 | 1   | 0.5                                    | 1 (positive outflow from the cleanroom and air dilution in corridors) | 0.75        |

It is now possible to obtain a risk rating by assessing each identified source and assigning risk scores to the four risk factors. Shown in **Table 2** is the first line of such a resultant assessment with a set of assigned scores and the risk rating obtained.

From the risk rating values, the importance of each source can be assigned a risk rating of ‘high’, ‘medium’ or ‘low’. For example, hazards that have a risk rating of:

- (a) 4 and over may be considered as ‘high’;
- (b) below 4, and over or equal to 2, as ‘medium’; and
- (c) less than 2 as ‘low’.

The designation of ‘high’ to a source does not mean that it has a high risk to the product, but within a list of possible hazards it is likely to be one with a higher degree of risk. Hazards with a ‘high’ or ‘medium’ risk can then be further considered with a view to reducing their risk. This can be

done by reducing the risk scores of one or more of factors A to D.

The list of sources, and their risk ratings, can then be used in subsequent parts of a risk management analysis based on HACCP, to determine whether adequate control methods are used, and if the risks or their control methods are adequately monitored. This can be done as indicated in **Table 3**, where the sources assessed previously are further considered. The HACCP system can then be further utilised to set monitoring schedules, appropriate action and alert limits, verify that the system works, prepare documentation and implement training<sup>5</sup>.

**Critical area risk assessment method**

The method of assessing risk described in the previous section is used to carry out an overall assessment of all microbial risks in all areas of the pharmaceutical

**Table 3.** List of sources with risk rating and consideration of a suitable control and valid monitoring methods

| Source to be controlled                          | Risk rating | Suitable control method             | Valid monitoring methods   |
|--|-------------|-------------------------------------|--|
| <b>1. Areas adjacent to critical area</b>        |             |                                     |  |
| 1.a. Air outside production cleanroom (corridor) | 0.75        | Adequate air ventilation conditions | 1. air supply volumes;<br>2. air pressurisation;<br>3. airborne particle conc.;<br>4. settle plates and airborne microbial sampling. |

production cleanroom suite. However, it will normally be found that the highest risk occurs in the critical area where the product is open to contamination. A more detailed risk assessment of the critical area may then be required to assess:

- (1) microbial contamination coming from contaminating surfaces, such as gloves or clothing, touching the product; and
- (2) the deposition of microbe carrying particles from the air.

It is necessary to assess these two routes separately, as the numerical values or descriptors used are basically different, and when steps are taken to minimise risks, the methods used will also be different.

### **Surface contact contamination**

Surface contact contamination can occur during the following stages of manufacture:

1. Transfers into the critical area;
2. Setting-up of production machinery;
3. Normal production;
4. Interventions.

The overall risk to the product by surface contact can be assessed by adding the risk from each of these stages, as shown in Equation 6.

### **Equation 6**

Risk by surface contact =  
risk from critical transfers + risk from setting up + risk during normal production + risk during interventions

When developing risk equations it is important to decide whether the risk factors should be multiplied, or added, as this has a substantial effect on the accuracy of the risk assessment. If risk factors are independent of each other, then they should be added together; this is the situation in Equation 6 where the risk of contamination in one stage of production has no direct influence on any other stage. However, if the risk factors are dependent on each other, then they should be multiplied. This is the case with the variables in the fundamental Equations 2, 3 and 4, as each variable (risk factor) is dependant on the others to determine the overall magnitude of the risk of contamination. One example of a dependent risk factor is time, as the time the product is exposed to contamination directly influences, or 'gears up', the degree of risk of the other risk factors. A practical way of deciding whether to add or multiply risk factors is to allocate zero to a risk factor, and find out if the result is the one that is anticipated.

It is now necessary to model the risk of each of the four stages given in Equation 6. It is not sufficient to list all of the factors thought to cause microbial risk during production, and multiply or add their risk scores together. If this is done, inaccuracies will emerge. For example, if two or more descriptors are used to describe the risk caused by the same fundamental variable, then the

importance of that variable will be exaggerated, especially if the risk scores are multiplied together. Numerical values, or descriptors, should therefore be chosen to measure or describe the variables in the surface contact contamination Equation 3, and combined as indicated in Equation 6.

The first variable in Equation 3 is microbial contamination on contacting surfaces. This variable is not used here, because many surfaces are not sampled for microbial contamination. If they are, they will be often found to be sterile, or any counts may be inaccurate because of the introduction of microorganisms during the sampling. However, if reliable surface concentration results are available then these can be included to improve the risk estimate. The second variable in Equation 3 is the transfer coefficient relating to the proportion of surface contamination that is transferred from the contaminating surface to the product. It is highly unlikely that any numerical values will be available for this variable, and descriptors should be used. The third variable is the area of the product that is contacted. If this varies between different stages of the process, then this variable should be incorporated to improve the risk analysis. However, it will be more common that the area will remain constant, and there is no advantage in including this variable. The variable of time, or frequency, which was not used as a risk variable in the overall risk assessment discussed above, is used here, as it is likely to be available, or can be determined. Time can be expressed either as the time the product is exposed to contamination, or the frequency of a contamination incident occurring. Utilising these assumptions, a simple risk equation that combines the surface contact contamination from the four stages of manufacture is given in Equation 7.

### **Equation 7**

Risk by surface contact =  $\alpha$  [no. of critical transfers] +  $\beta$  [setting up complexity] +  $\chi$  [operator involvement × complexity of critical area × no. of manipulations] +  $\delta$  [no. of interventions]

Where  $\alpha$ ,  $\beta$ ,  $\chi$  and  $\delta$  are weighting coefficients

It can be seen in this equation that the 'risk during normal production' variable indicated in Equation 6 now comprises of three risk descriptors. As these three descriptors are relatively dependent on each other, they need to be multiplied together. However, this combined risk score will be much greater than any of the other three stages. In addition, some stages are more likely to contribute more surface contamination e.g. interventions than other stages. These imbalances in the risk equation can be corrected by assigning weighting coefficients. These were shown in Equation 7 as  $\alpha$ ,  $\beta$ ,  $\chi$  and  $\delta$ , and examples of values that can be assigned are shown in Equation 8:

### **Equation 8**

Risk by surface contact =  $1 \times$  [no. of critical transfers] +  $1.5 \times$  [setting up complexity] +  $0.5 \times$  [body involvement × complexity of critical area × no. of manipulations] +  $2 \times$  [no. of interventions]

| Manufacturing Stage               | Risk factor                  | Risk score 0 = nil   | Risk score 1 = low                              | Risk score 2 = medium                            | Risk score 3 = high                      |
|-----------------------------------|------------------------------|----------------------|---|--|--|
| Transfers into the Critical Areas | Number of critical transfers | None                 | No more than 10                                 | No more than 30                                  | More than 30                             |
| Setting up Production Machinery   | Setting up complexity        | No set up activities | Not complex. Time to set up no more than 10 min | More complex. Time to set up no more than 30 min | Complex. Time to set up more than 30 min |
| Normal Production                 | Operator involvement         | None                 | Hands   | Hands and arms                                   | Body                                     |
|                                   | Complexity of critical area  | Not Applicable       | Simple  | Some complexity                                  | Complex                                  |
|                                   | Number of manipulations      | None                 | No more than 5% of time                         | No more than 50% of time                         | More than 50% of time                    |
| Interventions                     | Number of interventions      | None                 | No more than 5                                  | No more than 10                                  | More than 10                             |

It is now necessary to allocate risk scores to each risk factor in **Equation 8**. Risk scores are usually assigned by an aggregation of opinion from an expert committee set up to carry out this task. Some scoring systems use only numerical values, and these may range as high as from 0 to 10. However, it is unrealistic to expect that risk can be allocated in such fine divisions, and a range of no more than six levels is reasonable. It is also easier for a person to describe risk by one or two simple words that can be allocated a number e.g. not possible = 0, very unlikely = 1, unlikely = 2, possible = 3, likely = 4, very likely = 5, definite = 6. It is difficult to find a scoring system that is free of criticism but a simple 4-level system is used here i.e. nil - 0, low-1, medium-2 and high-3.

Given in **Table 4** is an example of surface contact risk factors and associated scores which could be applied to a manufacturing process. This should be modified to fit the process being investigated. It should also be noted that when constructing a risk score table the normal range of occurrence should be evenly spread to match the range of the risk scores. This is especially important if different stages, or phases, of production have to be compared. The

scores allocated are then used with Equation 8 to obtain a 'risk rating'.

Risk ratings can now be used to see where improvements are best made to reduce the risk. An example is given.

**Example:** *A cleanroom has a filling machine used for aseptic filling of vials that is located within a unidirectional flow workstation. Vials are fed to the point-of-fill through a hot-air depyrogenation tunnel. Product solution, stored in a sterilized holding tank, is fed to the filling machine through sterilizing grade filters and filled into the vials. Lyophilisation stoppers, transferred into the filling area, are partially seated onto the vials, which are accumulated into a mobile isolator, transferred into a sterilized freeze drier, and dried. The stoppers are then fully seated before being removed from the freeze drier for capping and crimping. The risk scores (bold) for each risk factor, and the risk rating, as calculated by Equation 8 for the four stages, are given in **Table 5**.*

**Table 5** indicates that the filling and stoppering stage had the highest risk assessment and the operations of this stage should be targeted to reduce the contact surface risk. Risk factors were addressed in the following manner:

| Manufacturing phase            | Transfers into the Critical Areas | Setting up Production Machinery | Normal Production                              | Normal Production     | Normal Production                | Interventions | Risk rating* |
|--------------------------------|-----------------------------------|---------------------------------|--|-----------------------|----------------------------------|---------------|--------------|
|                                | (A)                               | (B)                             | Number of manipulations (% of total time) (C1) | Body involvement (C2) | Complexity of critical area (C3) | (D)           |              |
| Vial washing and sterilisation | None score=0                      | None score=0                    | 1% score=1                                     | None score=0          | Simple score=1                   | 1 score=1     | 2.0          |
| Solution storage               | 3 score=1                         | 5 min score=1                   | 1% score=1                                     | None score=0          | Simple score=1                   | None score=0  | 2.5          |
| Filling and stopper placement  | 50 score=3                        | 35 min score=3                  | 3% score=1                                     | None score=0          | Simple score=1                   | 38 score=3    | 10.5         |
| Freeze drying and capping      | 150 score=3                       | 5 min score=1                   | One manipulation score=0.5                     | None score=0          | Simple score=1                   | None score=0  | 4.5          |

\* Risk rating = [1 x A] + [1.5 x B] + 0.5[C1 x C2 x C3] + [2 x D]

1. *The number of critical transfers: The vial stoppers were supplied in bags of 500. With a single batch size of up to 25,000 units, up to 50 critical transfers were required. As the stopper feed bowl had adequate capacity, the stoppers were supplied in bags of 1000, reducing the number of transfers by a factor of 50%.*
2. *Filling machine set up complexity and time: Control of filling volume was achieved via a time-pressure system with a high level of automated control. However, this required a complex aseptic assembly of machine parts that took on average 35 minutes to complete. Modifications to the pipework were undertaken, whilst maintaining its cleaning and sterilisation functionality. The resultant assembly operation utilised less parts, was less complex, and had an average assembly time of 9 minutes.*
3. *The number of interventions: Stopper-to-stopper adhesion, a consequence of the sterilisation process, resulted in feedbowl blockage, which then required manual removal with sterilised forceps. Modifications to the feed bowl, to automatically reject adhered stoppers, reduced the average number of interventions from 38 to 4.*

The above modifications reduced the risk rating of the filling and stoppering stage from 10.5 to 5.5.

The risk occurring during an individual manufacturing stage can, if required, be investigated in more detail. For example, the setting-up stage may be complex, with a large number of steps. The FMECA **Equation 1** given in the introduction of this paper can be used:

Risk = criticality of the occurrence x frequency of occurrence

The stage to be investigated should be broken down into individual steps. The risk scoring system should be set and scores allocated to the 'criticality' and 'time' for each step, these being multiplied together to obtain a risk rating. For illustration purposes, and using an arbitrary scoring system, a single step is shown in **Table 4**.

'Criticality' expresses the importance of a source, and it has been shown in Section 1 that it should be expressed in terms of the microbial concentration of the contaminating surface and the likelihood that these microbes are dispersed, transferred, and then deposited onto the product. As shown in **Table 6**, microbial concentration of the contaminating surface can be scored and an overall score used for the component of dispersion, transfer and deposition (these three components can also be scored individually if required). Time may be scored in relation to the time the product is exposed to contamination, or as contamination incidence frequency.

|  | Criticality   | x | Time                              | = | Risk rating   |
|--|---|---|-----------------------------------|---|---------------|
| Score of 'concentration of microbes (C)' | Score of 'dispersion x transportation x deposition (D.T.P)' |   | Score of 'time, or frequency (T)' |   | C x D.T.D x T |
| 1 x                                      | 3 x   |   | 5 =                               |   | 15            |

## Risk assessment of airborne deposition

### Airborne deposition using sampling results

**Equation 4** is the fundamental equation used to calculate airborne dispersion onto or into, pharmaceutical products. This equation has been shown to predict airborne contamination over a wide range of conditions during pharmaceutical production<sup>8</sup>. The variables required for solution of the equation are:

*Microbial deposition rate:* This can be obtained from the counts on settle plates exposed adjacent to the product. The results from settle plates are commonly reported as: number of colony forming units (cfus) settling onto a settle plate of a given area exposed over a given period of time. This is a deposition rate, but for our purposes must be presented in a more scientific way: cfus/cm<sup>2</sup>/hour is the most convenient. To achieve an accurate simulation of contamination, settle plates must be positioned adjacent to the exposed product and open only when contamination occurs; this can be done by opening and closing the plates when production starts and stops. However, the plate should remain open if production stops and an unplanned intervention is necessary when the product is still exposed. Settle plates 9cm in diameter (64 cm<sup>2</sup> in area) are normally exposed for four hours, although larger 14 cm diameter plates (154cm<sup>2</sup> area), exposed for the same period, will give more accurate counts. Settle plate counts taken in cleanrooms often give a series of zero results, with an occasional positive. This lack of sensitivity can be improved by using the larger plates during a period of high sampling intensity, or combining results obtained from routine sampling results, and averaging them over a one or two year period.

It is necessary to check that dehydration of the microbiological medium in the settle plates, caused by air movement, does not reduce the count. Experience shows that as long as the plate is well filled with agar medium, and its exposure in unidirectional flow is not much over four hours, the resultant count is not significantly affected<sup>9</sup>. However, it is necessary to confirm this on an individual basis.

*Surface area:* The surface area of the product exposed to airborne contamination is required. As it is known that gravitational settling mainly governs the deposition of microbe carrying particles during pharmaceutical manufacturing<sup>7,10</sup>, only the horizontal area of the exposed product is required. This might be the area of the inner neck of a container, or the upwards-facing area of a solid product.

*Time product exposed:* The time the product is exposed to airborne microbial contamination is required. The total manufacturing time should not be used, but the average time a single product is exposed to the airborne environment. For example, the first product through a filling line may be exposed for only a few seconds, but the last one will be exposed for the total time of the process; the average exposure time is therefore 50% of the total process time. If a holding area e.g. turntable or hopper is loaded in batches

then the 50% time should be used for each batch rather than the total time. Any time the product is covered or protected from airborne deposition should also be taken into consideration.

Using these variables as indicated in **Equation 4**, the number of microorganisms deposited during a manufacturing operation can be calculated as shown in the following example of high airborne contamination:

**Example:** Containers are filled within a unidirectional flow workstation. Several settle plates are exposed for four hours in the area adjacent to the open containers. The average microbial count from the 14 cm diameter Petri dishes (154 cm<sup>2</sup> area) was found to be 1.

The deposition rate (no./cm<sup>2</sup>.hr) of the microorganisms can be calculated from the settle plate data i.e.

Deposition rate (no./cm<sup>2</sup>.hr) = average count on settle plate ÷ [area of Petri dish (cm<sup>2</sup>) × time plate exposed (hr)]

$$= 1 \div [154 \times 4] = 0.0016$$

The container has an inner neck area of 2 cm<sup>2</sup> and is open to contamination during filling for an average of 10 minutes. The number of microbes that would contaminate the product by depositing through the neck area is then calculated from **Equation 4**, although the units used are hours instead of seconds i.e.

No of airborne microbes deposited into product in a given time = deposition rate (no./cm<sup>2</sup>.hr) × exposed product surface area (cm<sup>2</sup>) × time of product exposure(hr)

$$= 0.0016 \times 2 \times [10 \div 60] = 5.4 \times 10^{-4}$$

It is a reasonable assumption that microbe-carrying particles will be deposited randomly throughout production<sup>11</sup> and therefore 5.4 containers in 10,000 will be contaminated.

Clearly, a contamination rate of about 1 in 2000 is unacceptable and must be improved. If the time the product is exposed, its surface area, or the airborne microbial concentration is reduced, then the contamination rate can be reduced; this can be calculated. If, for example, the filling time is reduced to 6 seconds, the airborne contamination of containers will be reduced 100 times to 5.4 containers in a million.

#### **Reduction of the risk of microbial airborne deposition**

The previous section demonstrates that the risk of airborne contamination is dependent on the microbial deposition rate, the exposure time, and the deposition area, and can be readily calculated. It may be possible, especially at the design stage, to reduce the exposure time and deposition area by redesigning the process. However, the possibility of reducing microbial deposition onto the product requires the assistance of risk assessment.

Pharmaceutical products open to microbial contamination will be located in a EU Grade A environment, as found within a separative air device such as a unidirectional airflow workstation or cabinet, and this will be located in a turbulently-ventilated EU Grade B or C background cleanroom area. Airborne contamination will then occur from two sources and routes. These are:

1. Air transferred from a Grade B or C cleanroom into the separative air device. The amount of undesirable transfer depends on the effectiveness of the separative device in preventing the entry of airborne contamination.
2. Microorganisms dispersed from personnel into the air within the critical area. The fundamental equations predict that this depends on how much of their body is within the critical area, the personnel's microbial dispersion rate, the effectiveness of their cleanroom clothing in reducing dispersion, and the time personnel is within the area.

A reduction in airborne contamination risk is best achieved by separate consideration of these two sources.

**Air transfer into the critical area.** Ljungqvist and Reinmuller<sup>12</sup> have described a method that can be used to determine the penetration of cleanroom air into an enhanced air device. They suggest, firstly, that the airflow round the area is investigated by smoke visualisation techniques to gain an insight into any problems. The area outside the enhanced air device is then seeded with a known concentration of small airborne particles, and the number of particles that penetrate into the critical area during simulated production, is measured. The ratio of particles found at the critical area to those seeded outside, is then calculated and is used as an indication of risk. The effectiveness of the enhanced air device in preventing penetration of outside contamination can then be investigated and its performance improved if required.

**Reduction in airborne microbial contaminants dispersed within the critical area:** It is necessary to return to the fundamental **Equation 2** to determine the best risk equation that can be used to assess risk. This is likely to be similar to **Equation 9**.

#### **Equation 9**

Airborne risk = [amount of personnel's body within area] × [effectiveness of clothing] × [proportion of time personnel are within critical zone during production]

The accuracy of the equation may be increased by assigning weighting coefficients to balance the contribution of risk, in the manner similar to that undertaken for **Equation 8**.

The effectiveness of clothing is an important factor. This can be ascertained by the use of a dispersal chamber<sup>13, 14</sup>, but this apparatus is generally not available outside the research laboratory. It may therefore be necessary to assess clothing in terms of the following descriptors:

- (a) how well the clothing envelopes the body;
- (b) the effectiveness of the clothing fabric in filtering the body's emissions [use of the pore diameter of interstices of the fabric, and the particle penetration through the fabric<sup>13, 14</sup>]; and
- (c) the prevention of body debris being pumped out from insufficiently sealed openings at the neck, face, cuffs and ankles.

A risk assessment should then be carried out by a method similar to that described in Section 3 and steps taken, where possible, to reduce the degree of risk by:

- (1) reducing the amount of a persons body within the critical area;
- (2) improving the effectiveness of cleanroom clothing and;
- (3) reducing the time within the critical area.

The effectiveness of the reduction of risk by use of one or both of these methods described above can be determined by settle plate sampling.

## Discussion

The use of risk management systems is well establish in the electrical and mechanical industries, but seldom used in managing microbial risk in aseptic pharmaceutical manufacturing. Various risk management methods are available but the HACCP system, being devised for the food industry, is probably the best system to use with microbial contamination in pharmaceutical cleanrooms.

A major component of risk management is risk assessment, where the degree of risk for each hazard is assessed. Described within this paper are risk assessment systems based on fundamental models of microbial contamination that allow the most effective risk assessment methods to be utilised and support the established FMECA method of assessing risk.

A method of risk assessment has been developed, firstly, to provide an overall assessment of contamination from all areas of the cleanroom. Secondly, a risk assessment method has been developed that focuses on the manufacturing process in the critical area and examines separately the surface contact and airborne deposition routes of contamination.

For surface contact contamination, using risk factors that have either numerical values or descriptors of risk, a risk rating can be calculated for each critical stage of a manufacturing operation or a multiphase manufacturing operation. By appropriate use of the values allocated to the risk factors, the step, stage or phase of manufacture with the highest risk can be identified and the risks addressed accordingly.

For airborne contamination, the fundamental equation used to calculate airborne contamination is well established and hence the associated contamination rate in the critical area can be readily calculated. Reductions in product exposure time and deposition area can, if necessary, be effected to reduce the microbial risk and overall contamination rate. However, a further risk assessment method will be required to assess and reduce

the risk from airborne contamination.

Manufacturing stages, or steps, with the highest risk ratings can, if deemed unacceptable, be modified or amended to reduce the microbial risk. At some stage, however, the question of what is an acceptable level of risk will be encountered. This may arise because further reductions in the risk rating are not possible, or significant capital expenditure and investment in new items of equipment is required. At this stage, agreement between manufacturing, engineering and quality assurance personnel, utilising the risk data and other relevant supporting information (e.g. ongoing environmental microbiological results, sterility test results, and product simulation test data) will be required, as well as consideration of the potential microbial risk to the patient. Utilisation of a risk management system will assist in this task.

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