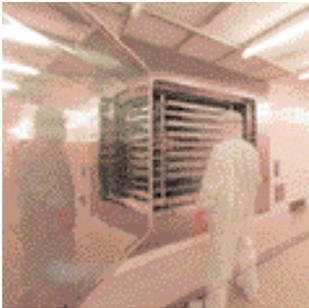


The LR Method in Critical Areas

Airflow Patterns and the Design of Aseptic Interventions

Bengt Ljungqvist and Berit Reinmüller*



DSM PHARMACEUTICALS, INC.

The authors discuss factual case studies involving the method for the limitation-of-risks (LR) method. This method can be used as an engineering tool in risk assessment work for the **identification, minimization, and evaluation of potential airborne risks**, and for the identification of adequate monitoring points.

Bengt Ljungqvist, PhD, is a professor of safety ventilation at Kungl Tekniska Högskolan and a consultant for pharmaceutical companies, hospitals, and laboratories. **Berit Reinmüller, PhD**, is a senior researcher in building services engineering at Kungl Tekniska Högskolan, SE 10044 Stockholm, Sweden, +46 8 790 7537, fax +46 8 411 8432, berit.reinmuller@byv.kth.se. She is also a consultant for the cleanroom industry.

*To whom all correspondence should be addressed.

Today's cleanroom technology offers tailor-made systems for most demands and as a result, unequipped rooms are not considered to be a problem. This is reflected in the requirement of European Union good manufacturing practices (EU GMPs) that a Grade B room must be 100 times cleaner at rest than during manufacturing conditions (1). However, the situation is completely different when cleanrooms are fully equipped and run with machinery, operators, processes, and sometimes temperature differences as well. Airflow patterns in clean zones can easily be disturbed by factors such as machine guarding (i.e., the protective barrier), equipment design, inappropriate component specifications, or necessary interventions. The effect of these problems becomes accentuated as inappropriate manual interference contributes to a higher potential risk of airborne contamination.

The predominant sources of contaminants within a cleanroom are people and machinery. Potential risk situations are created by the interaction among people, air patterns, and the dispersion of airborne contaminants, and are difficult to predict using common monitoring methods and computer simulations.

Today, FDA and regulatory agencies in the EU ask for documented studies about airflows in critical zones under dynamic conditions (1–3). Turbulence and stagnant air can act as a channel or reservoir for the accumulation of airborne contaminants (e.g., from an adjoining area with a lower classification). Preventing the entrainment of lower quality air into the critical clean zone is essential to achieve a high assurance of microbiological safety.

Risk assessment

Commonly accepted risk assessment methods can be applied to the assessment of airborne contamination of aseptic products produced in classified cleanrooms and clean zones. This is in agreement with the ISO standard regarding biocontamination in cleanrooms and with both EU and FDA GMPs (1–4). A structured risk analysis should be based on a documented system for checking and evaluating the process in terms of several parameters, taking into account the potential effect on product quality.

The key to this risk analysis is to understand the process, which in this case includes the clean zone and cleanroom and

Table I: Evaluation of various operator positions, with the measuring point near the vial infeed device (M1).*

Challenge region: along the hands and arms of the operator.	Number of particles in the critical area	Calculated risk factor	Incident frequency	Estimated total risk
Operator position A; simulating vial infeed	<10	$<10^{-4}$	Often	None
Operator position B; simulating vial infeed	120,000	$\sim 10^{-1}$	Often	High
Operator position A; simulating unloading of empty vials	20	$\sim 10^{-4}$	Often	Low
Operator position C; simulating removal of filled vials	20,000	$\sim 10^{-2}$	Relatively often	High

* Excerpt from case study.

their performance, as well as the manufacturing process and its vulnerability to airborne contamination. The risks of airborne contamination result from the potential for leakage of unfiltered air, entrainment of air from an adjoining area with a lower classification, and the accumulation of contaminants in turbulent and stagnant regions.

Methods and applications

To design and evaluate microbiologically safe aseptic manufacturing processes, several methods must be combined at different stages of process development. The common methods include computer simulations of airflow, airflow visualizations, airflow velocity measurements, installed filter leakage tests, the limitation-of-risks (LR) method, environmental monitoring methods, and process simulation (media fill).

Various methods are often used during different stages of the design and evaluation of aseptic processing equipment. For example, during the design and evaluation of an aseptic filling line, the following methods might be used:

- During the design phase, computer simulations can be used to predict airflow to compare different layouts under static conditions.
- During the construction phase, airflow visualization studies are sometimes used to illustrate main airflow patterns. Sometimes the LR method is used to compare and evaluate the aerodynamic performance of construction details in unidirectional air flow (UDF).

After installation, the function of the line and development of the process is started. To protect the critical zone, tailor-made sidewalls or restricted-access barriers must be constructed and evaluated. Here, air-movement visualization studies can provide valuable information given that the smoke or particles are emitted momentum-free, continuously, and under isothermal conditions. However, the visualization studies do not ensure microbiological safety against airborne contamination of components and products during production conditions. Instead, the protection efficiency of the sidewalls during dynamic conditions can be evaluated with the LR method. For example, the

LR method can be used to evaluate individual details such as the tools that must be constructed for equipment assembly and disassembly. The method also can be used to optimize safe aseptic interventions while detailed standard operating procedures (SOPs) are developed. In addition, monitoring locations can be evaluated with a modified LR method.

During the qualification phase, the function of the line, the safety of the process, instructions, and the monitoring system must be verified. Installed HEPA filters must be tested for leakage with an aerosol challenge. Classification of the critical zone and the surrounding cleanroom during operational conditions must be performed with regard

to both airborne particles and microbiological burden. Documented studies of airflow patterns also must be conducted. The final evaluation of total microbiological safety must be performed by means of repeated media fills and intensive environmental monitoring.

The method for the limitation-of-risks

For the design and evaluation of manual interventions, the LR method combines the visualization of airflows, a challenge test, and the calculation of a risk factor to provide a reliable procedure for assessing the microbiological risks of airborne contamination in clean zones in a systematic way. Ljungqvist and Reinmüller have described the method in detail (5–8).

In short, the LR method is performed in three steps. The first step is to visualize (e.g., by using a smoke technique) the main air movements and identify turbulent regions and critical vortices where contaminants can be accumulated or dispersed in an unpredictable way. The second step is the particle challenge test, which identifies potential risk situations. The particle challenge test involves placing the probe of a particle counter in the critical area where, during normal operations, the product is exposed, and taking continuous total particle counts while generating particles in the surrounding air (e.g., by using air current test tubes) to a challenge level of $>300,000$ particles of $\geq 0.5 \mu\text{m}/\text{ft}^3$ ($\sim 10^7$ particles/ m^3). These measurements should be carried out during simulated production activity. The third step is to evaluate the risk situation by calculating the risk factor, which is defined as the ratio between measured particle concentration (number of particles per cubic foot) in the critical region and the challenge level in the surrounding air. Because of the limitation of measurement accuracy at high concentrations, a value of $300,000/\text{ft}^3$ is used as a challenge level in all risk-factor calculations.

In the author's experience, when the risk factor is $<10^{-4}$ (0.01%) during the challenge test, it is expected that no microbiological contamination from the air will occur during normal operational conditions. Final media fills showing no contaminated units have verified these results.

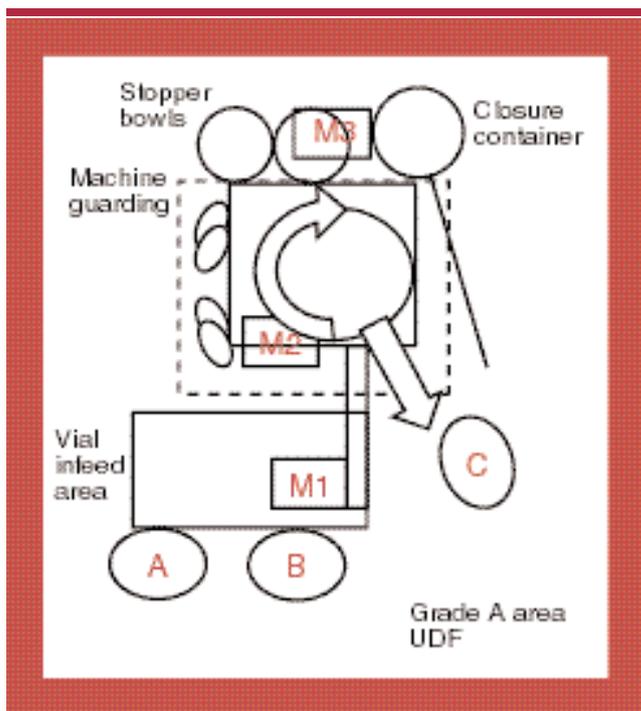


Figure 1: The layout of a filling line (from the case study, "Identification and Evaluation of Risk Situations"). The operator positions that were compared are marked as A, B, and C. The selected measuring points during the challenge tests are marked M1, M2, and M3.

Case studies from aseptic filling lines

Cleanroom requirements are demanding when stringent particulate control and aseptic conditions are required during manufacturing. This requirement applies in particular to filling lines located in HEPA-filtered unidirectional airflow. In such cases, turbulent regions and wakes can play critical roles by causing an accumulation of contaminants.

To predict potential contamination risks in real case situations, the use of the LR method provides valuable information

(8, 9). Through the years, more than 50 aseptic production lines have successfully been assessed with the LR method.

One case study involved a conventional filling line located under a large HEPA-filter unit with unidirectional airflow (Grade A, ISO Class 5, US Class 100, operational) with a background of Grade B (ISO Class 7, operational, US Class 10,000). The layout of the filling line is shown in Figure 1. The operator positions that were compared are marked as A, B, and C. The selected measuring points during the challenge tests are marked M1, M2, and M3. As part of the risk analysis, the total risk situation was assessed. The total risk is not only dependent on the calculated risk factor, but also on incident frequency. Particles were generated along the hand and arm of the operator performing manual interventions that might occur during normal production conditions. Results from the LR method are shown in Tables I–III. The results from the study were used to develop detailed SOPs for manual interventions on this specific line.

From the data in Table I, we can conclude that the operator's position influences the dispersion route of generated particles when measured where the vials enter the transport device (M1). High total risks were associated with operator positions B and C. As a result, the study clearly illustrated the lower risk connected to position A. It was not difficult to motivate the operator to use position A when unloading empty sterilized vials onto the belt. The verification of an adequate position for the operator when unloading the filled vials was performed in a similar way.

Table II shows the evaluation of manual interventions in the filling zone described in a draft SOP. The measuring location was inside the machine guarding near the actual filling zone (M2). Particles were generated along the hand and arm of the operator performing the manual interventions that could occur during normal production conditions. The results demonstrated that a longer tool was needed to remove broken vials. The instructions for the interventions ("adjustment of the filling volume" and "removal of a closure") implied no risk of airborne contamination. However, the suggested performance for the removal of a vial from the center of the transport wheel had to

Table II: Evaluation of manual interventions, with the measuring point within the filling zone inside the machine guarding barrier (M2).*

Challenge region: along the hands and arms of the operator.	Number of particles in critical area	Calculated risk factor	Incident frequency	Estimated total risk
Above infeed of vials; simulating removal of broken vial in transport device	490	$\sim 10^{-3}$	Relative often	Medium
Simulating adjustment of volume	<10	$< 10^{-4}$	Seldom	None
Simulating removal of closure in transport feed; door to guarding area open	<10	$< 10^{-4}$	Seldom	None
Simulating removal of vial from center of transport wheel	27,500	$\sim 10^{-1}$	Seldom	High
Outside guarding area; simulating feeding of stoppers into bowl	<10	$< 10^{-4}$	Often	None
Outside guarding; simulating feeding of closures into bowl	320	$\sim 10^{-3}$	Often	Medium

* Excerpt from case study.

Table III: Evaluation of manual intervention, with the measuring point along the edge of the stopper bowl outside the guarding area (M3).*

Challenge region: along the hands and arms of the operator.	Number of particles in critical area	Calculated risk factor	Incident frequency	Estimated total risk
Operator passing beside filling line, outside the UDF area	210	$\sim 5 \times 10^{-4}$	Often	Medium
Above adjacent stopper bowl; simulating feeding of stoppers into bowl	39,000	$\sim 10^{-1}$	Often	High
Above closure bowl; simulating feeding of closures into bowl	130	$\sim 4 \times 10^{-4}$	Often	Medium
Inside guarding; simulating adjusting of stoppering unit, door to guarding area open	47,000	$\sim 10^{-1}$	Seldom	High

* Excerpt from case study.

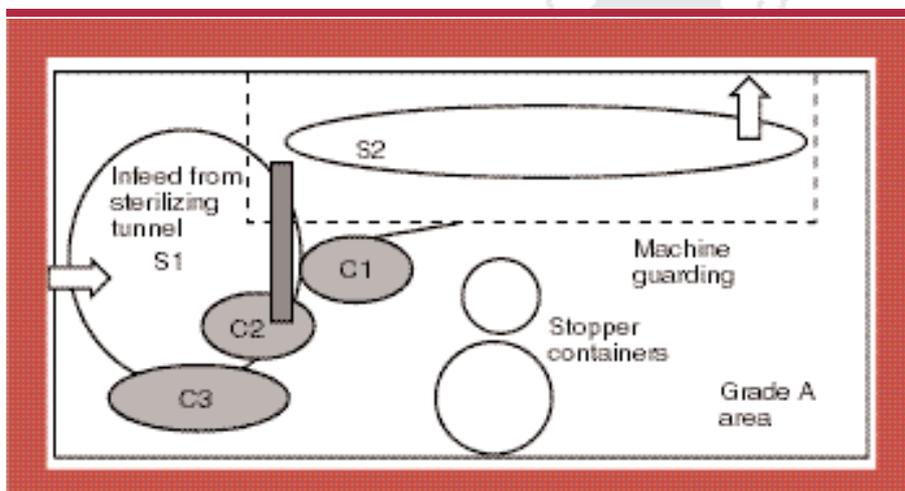


Figure 2: Evaluation of sampling locations. (Excerpt from case study).

be changed and instructions for a sufficient line clearance had to be added. It was also determined that conditions during the feeding of closures could be improved by prolonging the sidewall between the UDF area and the cleanroom.

Table III shows the evaluation of an operator passage close to the UDF area and manual interventions around the bowls for stoppers and closures. Entrainment of room air into the critical zone was illustrated. Suitable preventive actions were taken and evaluated. When filling stoppers into one bowl, particles were transported into the adjacent bowl; thus a risk situation was identified and instructions for the intervention as well as the design of the sidewall had to be adjusted to minimize the potential risk. It was determined that the intervention “adjustment of stoppering unit” should be avoided or performed with the aid of a special tool until the guarding was modified.

By classifying the total risk, preventive actions can be performed in a systematic way. More-severe risks are addressed before less-severe ones. When risk situations are identified, the frequency of the intervention must be questioned and the basic cause of the need for the intervention clarified. The number of

interventions during aseptic production should be as few as possible and carried out with the best tools available.

Excerpts from a second case study illustrate another application of the LR method—the evaluation of monitoring locations. Figure 2 shows the principle layout of a vial filling line in a clean zone with the selected monitoring locations (S1 and S2) and the tested challenge regions (C1, C2, and C3) marked. Here, a high risk factor indicates good agreement between monitoring location and risk situation. Table IV shows the results from challenge tests of the two chosen monitoring locations (S1 and S2) on the filling line shown in Figure 2.

Table IV illustrates the importance of choosing appropriate monitoring locations. The evaluation showed that location S1 did not give a false alarm when allowed interventions were performed and that a prohibited intervention caused traceable particle dispersion. Similarly, when a prohibited manual intervention was performed from within the filling zone, position S2 showed a clear response. If tests such as these are not conducted, a monitoring location could be chosen in such a way that no response to a critical disturbance or intervention is possible to detect. Knowledge about existing limitations in detection potential of the sampling locations increases the value of microbiological assessment.

Summary

Smoke studies alone can provide good information about air-flow patterns at rest, but are limited in identifying potential risks during activity. However, the LR method, with its smoke studies, challenge tests, and risk factor calculations, is essential to the detailed evaluation of processes involving human interventions and the development of appropriate equipment de-

Table IV: Evaluation of sampling locations.*

Sampling location challenge region	Number of particles at sampling point	Calculated Risk Factor	Response to incident
Sampling location above accumulation table (S1)			
C1: in front of guarding area, doors closed, outside filling zone; simulating operator intervention	<10	<10 ⁻⁴	Low
C2: on rotating accumulation table, simulating prohibited intervention, for removal of fallen vial	3,700	~10 ⁻²	Medium
C3: beside rotating accumulation table; simulating allowed intervention, for removal of fallen vial	10	~10 ⁻⁴	Low
	50		
Sampling location in filling zone (S2)			
C1: in front of guarding area, guarding doors closed, outside filling zone, simulating operator intervention	<10	<10 ⁻⁴	Low
C1: in front of guarding, outside filling zone, both guarding area doors open; simulating prohibited operator intervention	27,000	~10 ⁻¹	High

* Excerpt from case study.

sign. The LR method is an engineering tool that allows details to be evaluated and provides valuable information concerning weak links. By providing real time results, the method also offers results for educational purposes and motivates the operator to act and work in a correct manner.

The use of challenge-test methods for the validation of cleanroom processes such as sterilization processes, sterile filtration methods, and HEPA-filter installations are well established procedures. The microbiological assessment of potential hazards of airborne contamination can, in a similar way, be challenged and validated with the LR method. To understand the microbiological assessment of airborne contamination, knowledge regarding the interaction between air movements and the dispersion of contaminants is crucial. The awareness of the limitations of the sweeping action of air for the removal of contaminants is improved by informative smoke visualization studies, and the real-time results of the challenge test.

When potential risk situations are identified (e.g., manual interventions), eliminating intervention is the first step. The second step is to minimize the negative influence of the intervention. Risk situations can be eliminated in more than one way (e.g., machine function improvements, tighter component specifications, or adequate guiding rails). Minimizing the risks associated with interventions can be made by the use of adapted tools, detailed and verified instructions, and operator training.

When monitoring cleanrooms and clean zones and their processes, it is vitally important to identify potential hazards and to select sampling locations that respond to changes in the critical regions and known risk factors. Systematic risk analysis that identifies critical control points and establishes monitoring locations in a rational manner can improve the quality of cleanroom monitoring and the microbiological assessment of aseptic processes.

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