Airflow Visualization Studies
The Impact of Annex 1 (2022) on Sterility Assurance

Introduction

Understanding critical air patterns and their impacts is paramount to controlling an environment and is a crucial activity in adhering to the relevant regulations. Scientific demonstration is the basis of pharmaceutical GMP regulatory compliance, and the best way to demonstrate air patterns is through airflow visualization studies (also known as smoke studies). If unidirectional airflow (UDAF) is used as a tool for mitigating the risk of contamination, a company must demonstrate its effectiveness through such studies. Considered a pivotal aspect of the quality by design (QbD) of a filling line for years, airflow visualization studies have become one of the key aspects of a company's contamination control strategy as these studies take a central new role in the new Annex 1 revision from 2022.

Airflow visualization studies, given their wide range of application, affect several topics of Annex 1 (2022):

1. Cleanroom and clean air equipment qualification
2. Environmental monitoring program
3. Training and qualification of personnel
4. CCS (Contamination Control Strategy)

1. Cleanroom and Clean Air Equipment Qualification

As stated in Section 4.25 of Annex 1, airflow visualization is one of the qualification requirements described in Annex 15.

> *"4.25 Cleanroom and clean air equipment qualification is the overall process of assessing the level of compliance of a classified cleanroom or clean air equipment with its intended use. As part of the qualification requirements of Annex 15, the qualification of cleanrooms and clean air equipment should include (where relevant to the design/operation of the installation):*
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> *i. Installed filter system leakage and integrity testing.*
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> *ii. Airflow tests - volume and velocity.*
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> *iii. Air pressure difference test.*
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> *iv. Airflow direction test and visualisation.*
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> *v. Microbial airborne and surface contamination.*
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> *vi. Temperature measurement test.*
>
> *vii. Relative humidity test."*
>
> *Annex 1 (2022)*

Studies should be conducted both in operation and at rest. The outcome of the two studies is different and should be applied in different ways:

- Studies ***at rest*** are intended to prove that the airflow in the Grade A /ISO 5 areas is unidirectional and always flows to the areas with a lower level of cleanliness. They are also important for verifying that the airflow properly invests critical surfaces without risky backflows (traveling on less clean surfaces and going back) and also checking the absence of ingress from lower-grade to higher-grade areas. Therefore, the outcome of these studies is an assessment of the correct design of the cleanroom or filling line.
- Studies ***in operation*** are intended to prove that under operating conditions there are no equipment or personnel aseptic interventions that would disrupt the ISO 5/Grade A unidirectional airflow directed toward critical areas where critical surfaces, critical materials, and/or product are exposed. These studies are very important in assessing the impact of process operations.

The role of airflow visualization studies is further explained in Section 4.15 in the Annex 1 2022 revision.

> *"4.15 Airflow patterns within cleanrooms and zones should be visualised to demonstrate that there is no ingress from lower grade to higher grade areas and that air does not travel from less clean areas (such as the floor) or over operators or equipment that may transfer contamination to the higher grade areas. Where unidirectional airflow is required, visualisation studies should be performed to determine compliance, (see paragraphs 4.4 & 4.19). When filled, closed products are transferred to an adjacent cleanroom of a lower grade via a small egress point, airflow visualization studies should demonstrate that air does not ingress from the lower grade cleanrooms to the grade B area. Where air movement is shown to be a contamination risk to the clean area or critical zone, corrective actions, such as design improvement, should be implemented. Airflow pattern studies should be performed both at rest and in operation (e.g. simulating operator interventions)."*
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> *Annex 1 (2022)*

In order to be able to intervene in the design of the cleanroom or clean air equipment in time, it is possible to predict airflow patterns through CFD (Computational Fluid Dynamic) studies. The objective of CFD studies is to gain highly detailed results with regard to the flow through both time and space and thus detailed information about the flow fields. Through these tests, it is possible to determine the air speed and pressure distribution in the work area and verify flows while the process is still in a QbD phase which means that the design of the filling lines can be modified.

While CFD studies can provide valuable information earlier on in the design process, smoke studies are the tests most frequently used by pharmaceutical companies to conduct airflow visualization studies. This type of study is conducted during the initial qualification of the cleanroom/ filling line, and it must be repeated if there are changes in validated/qualified conditions that could impact airflow (such as changes in process, operations/interventions, equipment design, or if relevant regulations change). Through a risk-based approach, it is also possible to define a periodic frequency of retesting.

FIGURE 1 SMOKE STUDY RECORDING

Smoke studies have specific requirements that must be met. The installation of smoke feeders must provide proper visualization of air flows. For this reason, it is important that the smoke feeders are positioned perpendicular to the UDAF. The use of flexible hoses with extensions allows larger areas to be covered by starting the smoke release directly from the point where the air flows out of the filter. Smoke generation must be enough to make any air turbulence clearly visible and display the direction of the air. At the same time, however, smoke generation should not be so intense as to excessively reduce the visibility of the area.

All activities conducted during the study must be properly documented and video recordings showing the activity performed must be available in addition to the protocol and the study execution report as written in Section 4.15.

> *"4.15 Video recordings of the airflow patterns should be retained."* ***Annex 1 (2022)***

Recordings should be conducted with an HD camera and, if necessary, with multiple cameras from different perspectives. The videos should clearly show the activities conducted by the operators as well as the impact of these activities on airflow; the positioning of the camera(s) should be at the right angle to capture the activities being conducted. To successfully record smoke studies, it is often necessary to use black backgrounds to create the appropriate contrast and additional light sources. If there are areas divided by curtains or barrier systems (Isolator or RABS doors), it may be necessary to film from inside those areas as well as from outside. Note that a good cameraman is not enough; the video director(s) must have strong knowledge of the process and expertise in sterility assurance. The presence of an experienced team during the execution of the recording can lead to great time (and cost) savings, preventing the videos from having to be repeated in the future.

One of the most common mistakes is to film only part of the activities related to the process. Filming should take as long as necessary to fully record the simulated activities including set-up, monitoring, material transfer, and personnel flows.

Airflow visualization is part of the justification needed in a CCS as outlined in Section 9.22 below.

> *"9.22 Where aseptic operations are performed, microbial monitoring should be frequent using a combination of methods such as settle plates, volumetric air sampling, glove, gown and surface sampling (e.g. swabs and contact plates). The method of sampling used should be justified within the CCS and should be demonstrated not to have a detrimental impact on grade A and B airflow patterns."*
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> ***Annex 1 (2022)***

The output of an airflow visualization study can also be crucial for the validation of VHP cycles within smaller environments such as isolators. In fact, during the definition of the positions of the chemical indicators (CIs) and biological indicators (BIs) for the validation of the bio-decontamination cycle, one of the factors to be considered is airflow. The visualization of airflow makes it possible to determine which areas might be most critical. In combination with the analysis of the process, two aspects that can determine the criticality of the area are the presence of turbulent motions or the presence of areas that are difficult to reach for the bio-decontaminating agent.

Also in relation to validations, it is very important to note that there is a close correlation between air visualization studies and aseptic process simulation (APS). The outcome of air visualization studies may be one of the factors that must be considered in the risk assessment of the different interventions to be simulated.

As previously stated, the activities related to the study should be listed within a protocol, and responsibilities for execution and review should be accurately described. All necessary materials, equipment, and acceptance criteria (discussed in this document) must also be reported. The document should include a master list of the operations

to be simulated as well as an accurate description of the process and the area impacted by the study.

The results of the study should be recorded within a report where the outcome will be discussed. If acceptance criteria are not met, it is essential to conduct an investigation to identify the root cause and define a corrective action preventative action (CAPA) plan. CAPAs with low impact are often changes in operating procedures or the introduction of tools during aseptic manipulations. In worse cases, a change in the cleanroom or filling line design can be necessary.

2. Environmental Monitoring Program

The new Annex 1 revision specifies that it must be verified that monitoring systems and related activities do not have a negative impact on air flows through air visualization studies. This means that studies must be conducted with the systems installed and all monitoring activities must be simulated and recorded. The outcome of the air visualization studies should be considered when establishing monitoring positions.

> *"4.15 … The outcome of the air visualisation studies should be documented and considered when establishing the facility's environmental monitoring programme."*
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> *Annex 1 (2022)*

The outcome of air visualization studies needs to be assessed prior to defining monitoring locations, and at the same time, the impact of systems needs to be assessed on air flows. Do the studies need to be repeated before and after the installation of monitoring systems? The simplest answer is yes, but it is not the easiest choice to implement.

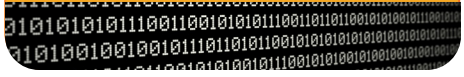
One possible workaround, as described in the previous section, is CFDs; they can be a very useful tool for predicting air flows and conducting an initial analysis prior to the physical installation of systems.

Airflow visualization studies are very important though, and there are several outcomes of the air visualization studies that need to be considered when establishing a monitoring plan. The presence of air turbulence can facilitate the transfer of contaminants in the area; contaminants present on surfaces even marginal to the process can be transported to critical areas due to turbulent air motions. In addition, an in-depth analysis of air visualization studies (especially in operation) can highlight other risk situations such as a possible entry of air from dirtier to cleaner areas or scenarios where the same air flows over the operator's garments and then subsequentially over other sterile surfaces. Actions should be taken if these outcomes arise in a study of the airflow. Where action cannot be taken by changing the design of the cleanroom (or clean air equipment) or where the problem cannot be solved by changing operational procedures, monitoring can be one of the risk mitigation tools to be adopted.

Therefore, in risk assessments for the definition of the monitoring plan, air visualization studies are a key parameter to be considered.

An example would be a process where operators are actively involved near or inside critical areas: air visualization studies can help identify which area of the sterile gown should be monitored following each intervention.

See the Risk Assessment as a Process Quality Assurance Tool by PMS for more details about risk management!



3. Training and Qualification of Personnel

As outlined in the previous sections, one of the great advantages of air visualization studies is making the impact of production activities on airflow visible. The visualization of the impact of one's behavior is what makes training of any kind effective. In sterility assurance, it is not always easy to increase the sensitivity of the operators involved in the processes especially if their level of knowledge on the subject is not very high. This is precisely why the new Annex 1 requires considering the review of the air visualization studies as part of personnel training and qualification as shown in Section 7.18 below.

> *"7.18 Activities in clean areas that are not critical to the production processes should be kept to a minimum, especially when aseptic operations are in progress. Movement of personnel should be slow, controlled and methodical to avoid excessive shedding of particles and organisms due to over-vigorous activity. Operators performing aseptic operations should adhere to aseptic technique at all times to prevent changes in air currents that may introduce air of lower quality into the critical zone. Movement adjacent to the critical zone should be restricted and the obstruction of the path of the unidirectional (first air) airflow should be avoided. A review of airflow visualisation studies should be considered as part of the training programme"*
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> *Annex 1 (2022)*

One tool that can be integrated in this process is the use of virtual reality (VR) for the simulation of risky operations. VR would allow operators to visualize the impact of different production activities on airflow without possible negative consequences on the process and could provide considerable savings in time and resources.

To date, however, the most widely used tool for training is the review of smoke studies conducted both in operation and at rest. Reviewing smoke studies in at-rest conditions and in operation allows operators to appreciate the difference in airflow as a result of the different activities conducted. The videos, if the study is conducted correctly, can be good training on the correct execution of aseptic techniques which should be combined with practical simulations. For review, it can also be useful to simulate some of the most common errors during the visualization studies for purely training purposes; some examples might be the interruption of the "first air" (i.e., Grade A air exiting the HEPA filters in a unidirectional manner) directed towards critical areas where sterile material and products are exposed, the non-use of tools, or the prolonged opening of the doors of a filling machine.

For the reasons described above, the review of smoke studies should be one of the steps in the qualification of operators to enter and perform activities inside the cleanroom.

4. Contamination Control Strategy (CCS)

As outlined throughout this paper, performing air visualization studies is one of the key steps in building a contamination control strategy (CCS). This is because the knowledge of the process also passes through the execution of this activity, but not only that, the new Annex 1 revision is very clear that the training and qualification of personnel are key aspects to be evaluated in our CCS.

During the drafting of the CCS, the recordings of the performed air visualization studies must be reviewed (along with other relevant materials) to highlight any gaps in the process. The correct execution of air visualization studies can be an important mitigation tool for some risks, especially for risks related to activities that require operators handling (e.g., set-up of sterilizing filters or sterile machine components) and exposure to critical areas.

It is good practice, therefore, to define internal guidelines for the correct execution of these studies and to repeat them if the need is highlighted at the end of the drafting of the CCS.

How Can PMS Help You With The Execution of Air Visualization Studies?

As experts in sterility assurance, the PMS Advisory Team can support companies worldwide in all phases of smoke studies. Support can be conducted remotely through training, drafting of the documentation for the study execution, and review of records. Alternatively, onsite support can also be provided; the advisor can actively participate in the execution of the study in all its phases, offering a sterility assurance expert point of view during simulations and leading the operators in the correct execution of aseptic techniques.

What is the Advantage of Calling a PMS Advisor?

Smoke studies are often time-consuming activities that require a great effort from pharmaceutical companies from the use of personnel involved in the activities to long shutdowns of entire cleanrooms. The design and execution of these studies also requires specific sterility assurance skills that are not always available in-house. The help of a PMS advisor can make the process much faster and consequently less expensive. The PMS team's experience in the field can help to avoid deviations by the authority that would force the company to repeat the studies, or worse.

Conclusion

Air visualization studies are a critical aspect in the new Annex 1 revision and the subject of numerous comments from regulatory agencies in recent years.

To be in line with regulatory requirements within the timeframe required by Annex 1, it is important to accurately describe instructions for the execution of these studies within a standard operating procedure, review existing recordings, repeat the studies if necessary, and update the site contamination control strategy accordingly. Incorrect execution of air visualization studies results in an incorrect assessment of process-related risk, challenging the entire qualification of cleanrooms/clean air equipment and monitoring plan.

The design of these studies requires the collaboration between different departments within the company such as engineering, manufacturing, QA, and sterility assurance. Given their impact on the site CCS, the presence of a sterility assurance expert is required from the early stages of the studies to their development in the field and analysis of results. Videos from these smoke studies are raw data and should be treated as such. This means that it is important to pay particular attention to data integrity.

To recap, the necessary parts to meet an inspector's expectations are a defined flow for the management of these studies (in which all relevant departments are actively involved), transparency in content, recordings conducted according to the principles defined in this document, and proper use of the raw data for training and contamination control risk assessments.

References

[1] Annex 1, Manufacture of Sterile Medicinal Products -The Rules Governing Medicinal Products in the European Union Volume 4 EU Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use - 22.8.2022 C(2022) 5938 final

[2] US CFR Title 21, Part 211.

[3] PIC/S PE 009-16, Annex 1 (2022)

[4] FDA Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing (2004)

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Luca is a Doctor in biological sciences with experience in pharmaceutical microbiology who is currently advisory specialist in the global sterility assurance team of Particle Measuring Systems. He started his career in the microbiological quality control laboratory, before moving into sterility assurance. He has been involved in the risk management and validations of multiple production processes and new lines starting from the quality by design phase. At PMS, he works closely with many pharmaceutical companies and OEMs around the world, becoming a quality risk management expert. He also provides training and speeches for ISPE (International Society for Pharmaceutical Engineering), AFI (Associazione Farmaceutici dell'Industria), PDA (Parental Drug Association) and for Italian universities.