AIRFLOW VISUALIZATION: STANDARDS, REGULATIONS AND COMMON MISUNDERSTANDINGS

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AIRFLOW VISUALIZATION FOR CONTAMINATION CONTROL

Too often Airflow Visualization Studies are approached only as a regulatory requirement and not as a contamination control tool. Airflow visualization studies are an extremely important tool in assessing the physical contamination control effect of air patterns and should be used to identify risk based environmental sampling locations.

It is important that in medical product cleanrooms, dislodged particles should not be redeposited on products, personnel, or work surfaces. Because human beings are a constant source of particulate and microbial contamination, a pragmatic and scientific approach must be deployed to prevent contaminated medical products. Airflow visualization as applied to the science of contamination control provides valuable information in terms of assessing contamination risk. Understanding air patterns in cleanrooms inclusive of equipment and personnel movements is a fundamental component of holistic contamination control.

CONDUCTING AIRFLOW VISUALIZATION STUDIES: METHODS AND TECHNIQUES

The necessity of having a broad range of controls such as cleanrooms, barrier systems, sterilization, sterile gowning, aseptic practices, and risk based environmental monitoring is due to the seriousness contamination poses to patients. It is important to note the impossibility of maintaining a sterile cleanroom environment that has humans working in it for any period of time. Even with the most technologically updated facility designs and rigid sterile cleanroom gowning practices, human, and environmental contamination is a constant threat to aseptically produced products. It is difficult to fully eliminate microbial and particulate contamination in the entire operating cleanroom, however, using all these controls and the use of robust, appropriately clean airflow, contamination can be prevented from reaching products and product contact surfaces for the duration of aseptic operations.

Because no amount of testing and monitoring can guarantee a contamination free manufacturing environment, airflow visualization should be utilized to optimize and qualify the contamination control effect of the airflow under all operating conditions.

As regulatory bodies are expected to review airflow visualization videos, it is important to work out any shortcomings in the system before attempting the documented “in situ air pattern analysis”.

The actual (or physical) contamination control effect of cleanroom airflow can only be properly understood when it is visually represented. From this visual representation and analysis:

- Engineering can optimize the cleanroom, RABS, Isolator, or other barrier system design and integration of these systems into the cleanroom. Poor equipment placement and integration can lessen a cleanroom’s ability to remove contamination. Other factors such as equipment exhausts or cooling fans can significantly alter air patterns and be a possible channel for contamination.

- Manufacturing can optimize operator positions, movements, and sequences to reduce the risk of human generated contamination near product or product contact surfaces. Videos can be used for operator training and SOP development.

- Quality Control and Validation can review changes and create a suitable means of

documenting static and dynamic in situ air pattern analyses once the optimization of the cleanroom or clean zone and the operator's movements have been defined.

WHAT ARE AIRFLOW VISUALIZATION STUDIES (AVS)?

Airflow visualization is the science of making airflow patterns visible for analysis and is useful in contamination control. Because air is transparent, airflow patterns are invisible to the naked eye. The most common method to make air patterns visible is the Tracer Particle Method. The tracer particle method utilizes the addition of millions of tracer particles into the air stream being tested. This smoke-like cloud of tracer particles makes the air patterns visible, allowing the analysis of the physical (actual) airflow patterns against design and operational requirements. The term "Smoke Study" is often used synonymously for airflow visualization as originally these studies utilized smoke sticks, cigarettes, incense, and powders.

The accuracy of these studies and the conclusions obtained, is dependent upon multiple factors. The methodology, equipment and the material used, can impact the accuracy of the airflow visualization studies. Inaccurate airflow visualization studies have created incorrect conclusions regarding airflow in medical product cleanrooms. Critical areas that are reported to have suitable unidirectional airflow, actuality have vortices or other adverse airflow patterns. These adverse air patterns can act as a channel or reservoir for contamination. This has led to contaminated products, failed media fills, sterility assurance failures, 483 observations, warning letters, import notifications and negotiated facility closures.

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TECHNOLOGY FOR AIRFLOW VISUALIZATION STUDIES

The Tracer Particle Injection Method typically used in cleanrooms involves the observation and recording the behavior of tracer particles that are injected or diffused into the air stream being tested.

The accuracy of the air pattern analysis is dependent upon:

- Tracer particles faithfully following the air patterns.
- Tracer particles remaining visible long enough to allow for the analysis of the area being tested.
- Location of the tracer particle injection.
- Method the tracer particles are injected into the air patterns being tested.

Various conditions (e.g., tracer particle size, temperature, composition, vapor pressure, gravity) may influence the tracer particle behavior and stability. These conditions influence the behavior of the tracer particles, causing them to deviate from the actual airflow patterns being tested. Tracer particles that settle or disappear rapidly may not accurately demonstrate air patterns in critical areas. This has resulted in incorrect conclusions related to airflow visualization studies. Various systems and equipment are used for performing airflow visualization and creating the tracer particles with varying levels of accuracy.

Examples of equipment used for airflow visualization

- Ultrasonic, water based "Cleanroom Foggers". These systems use an ultrasonic transducer or series of transducers with WFI, distilled, purified, or deionized water.

The transducer vibrates at an ultrasonic frequency creating tiny water droplets (tracer particles) that are propelled via a fan into the area of interest. These tracer particles are unstable and expand or coalesce creating larger particles that are heavier than the air. Tracer particles dispersed from the output hose or manifold of these systems range between 5-25 µm in size. Because gravity influences the behavior of these tracer particles, these systems cannot reliably visualize dead air spaces or other adverse airflow patterns (see figure 1). Condensation on nearby surfaces and fogging of windows in RABS and Isolators may occur when using these systems.

- CO_2 (dry ice) and water based "Cleanroom Fog Generators". These systems use WFI, Distilled, purified or DI water and Carbon Dioxide to create tiny water droplets (tracer particles) that are propelled via a fan into the area of interest. These tracer particles are unstable and evaporate rapidly. Tracer particles dispersed from the output hose or manifold of these systems range between 5-10 µm in size. Because gravity influences the behavior of these tracer particles and they rapidly evaporate, these systems cannot reliably visualize dead air spaces or other adverse airflow patterns in critical areas.

CONDUCTING AIRFLOW VISUALIZATION STUDIES: METHODS AND TECHNIQUES

- Nitrogen and water "Ultra Clean Cleanroom Fog Generators". These systems use deionized water and nitrogen to create tracer particles. These tracer particles however are heavier than the air and evaporate rapidly. Tracer particles dispersed from the output hose or manifold of these systems are approximately 2 µm in size. Because gravity influences the behavior of these tracer particles, these systems cannot reliably visualize dead air spaces or other adverse airflow patterns (see figures 2,3).

- Glycol and water based "Tracer Particle Generators". These systems vaporize a propylene glycol and water-based solution that when suitably diffused create sub-micron tracer particles approximately 0.3µm in size. These tracer particles are neutrally buoyant and stable (see figures 4, 5). Tracer Particles created from these systems, stay visible long enough to evaluate the area being tested. The glycol and water technology utilized is the same as artificial smoke generators used in movie, stage, television, and fire safety training. The main component, propylene glycol, is non-toxic, anti-microbial and is used in pharmaceutical formulations (eye-drops, injectables), food manufacturing (as a preservative), personal care products (shampoos, conditioners, lotions), and e-cigarettes (creating the smoke like effect).

Tracer Particles used for in Airflow Visualization Studies

For consistent and repeatable results, the tracer particles (smoke cloud) must be neutrally buoyant, stable, and suitably diffused or injected into the air stream being tested.

Neutrally Buoyant Tracer Particles

Neutrally Buoyant describes the behavior of Tracer Particles when they are diffused into an area with no apparent airflow. The cloud of tracer particles should not settle or rise rapidly after being released into an area with no airflow. This allows for the detection and recording of adverse airflow such as dead spaces, turbulence, refluxing air or vortices (eddy currents). Tracer particles that settle rapidly cannot detect if HEPA filters are turned off (see figure 2), a significant requirement for accurate airflow visualization studies.

Water based (Ultrasonic, CO_2, Nitrogen) "Cleanroom Foggers" produce a fog (of tracer particles) that is not neutrally buoyant. These systems are sold based upon the cleanliness of the fog and not the ability to map the actual air patterns. Unfortunately, the use of these systems has created problems for pharmaceutical manufacturers as these systems cannot reliably detect dead air spaces, vortices, and air exchanges from door openings in critical areas. These water-based foggers can make non-cleanroom areas such as meeting rooms appear to have unidirectional airflow (see figure 1).

Figure 1: Ultrasonic and water "Cleanroom Fogger" in a meeting room without airflow.

The smoke sinks, giving the false impression of unidirectional airflow.

Figure 2: Nitrogen and water "Cleanroom Fogger" Beneath HEPA filters. Note: HVAC is Off

The smoke sinks, giving the false impression of unidirectional airflow.

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Figure 3 Nitrogen and water "Cleanroom Fogger" beneath a florescent light (dead zone) the smoke sinks, giving the false impression of unidirectional airflow

Figure 4 Glycol-based Tracer Particle Generator Note: Beneath HEPA filters HVAC is Off

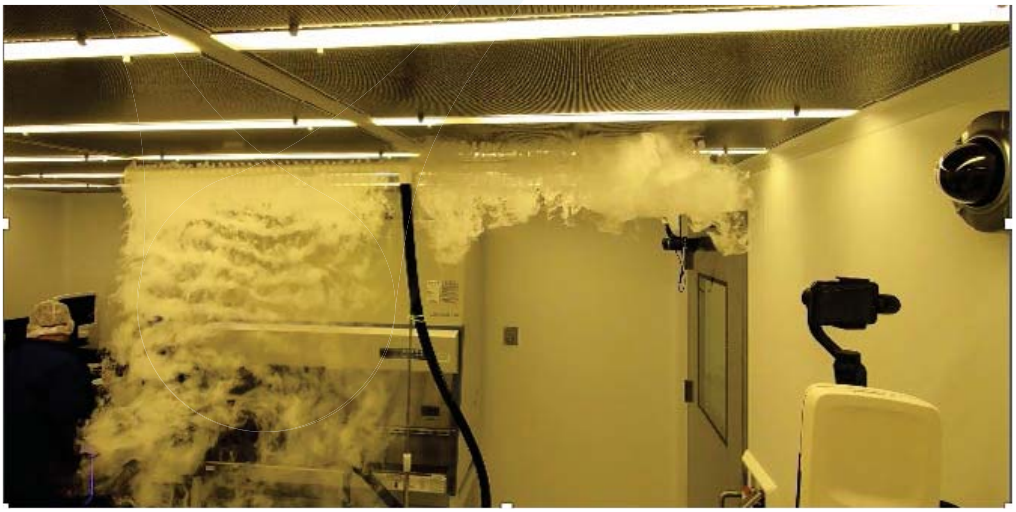

Figure 5 Glycol-based Tracer Particle Generator testing two HEPA filters in a unidirectional flow cleanroom. The filter on the left demonstrates unidirectional airflow, the filter on the right is turned off.

Stable Tracer Particles

The Tracer Particles used for accurate AVS should be Stable (visible long enough to evaluate the area being tested). In order to accurately observe and record the air patterns by observing tracer particles, these particles must remain visible long enough to record and document the air patterns being tested. Tracer particles that are unstable

and evaporate too rapidly cannot accurately characterize the "Contamination Control Effect" in cleanrooms and controlled environments. These particles simply do not remain visible long enough to observe mixing of air in non-unidirectional flow areas.

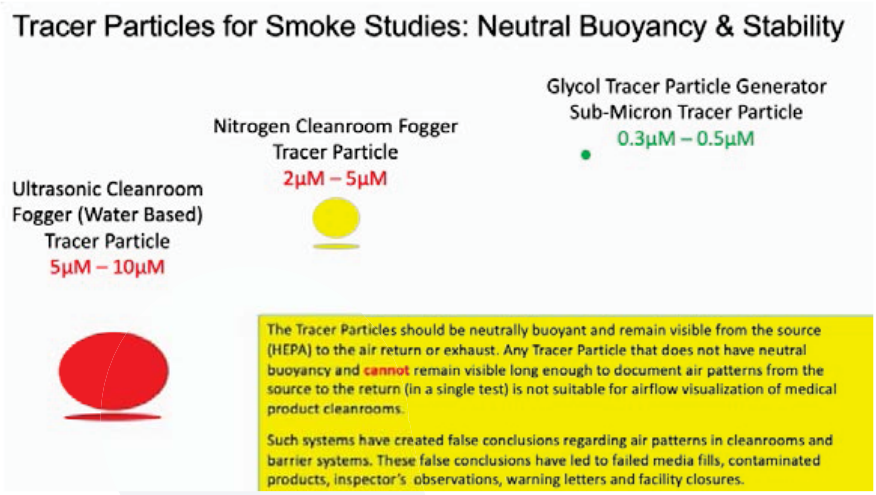

Figure 6: Tracer Particles for Smoke Studies: Neutral Buoyancy and Stability

Tracer particles must be suitably diffused into the air stream

For accurate airflow visualization it is important that the tracer particles are introduced without altering, disturbing, or overpowering the air patterns being tested. Per the FDA guidance "In situ air pattern analysis should be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions."

Referring to the definition of the word "In situ": meaning situated in the original, natural, or existing place or position, undisturbed. In order to deliver the tracer particles into the critical area the use of; tubing, manifolds, stands, suctions cups, cable ties or tripods may be necessary. Additional test personnel operating a wand or tube to inject the tracer particles through a partially opened door, should be avoided if possible.

The use of a tracer particle dispersion manifold should be considered to best deliver the tracer particles to the critical area being tested. Manifold design and orientation can influence the accuracy of the airflow visualization.

Manifolds with a slotted or single row of orifices along the length of the manifolds (see Figure 7.) must be orientated to not overpower the air patterns being tested and give the false impression of unidirectional airflow when the actual air pattern is something different.

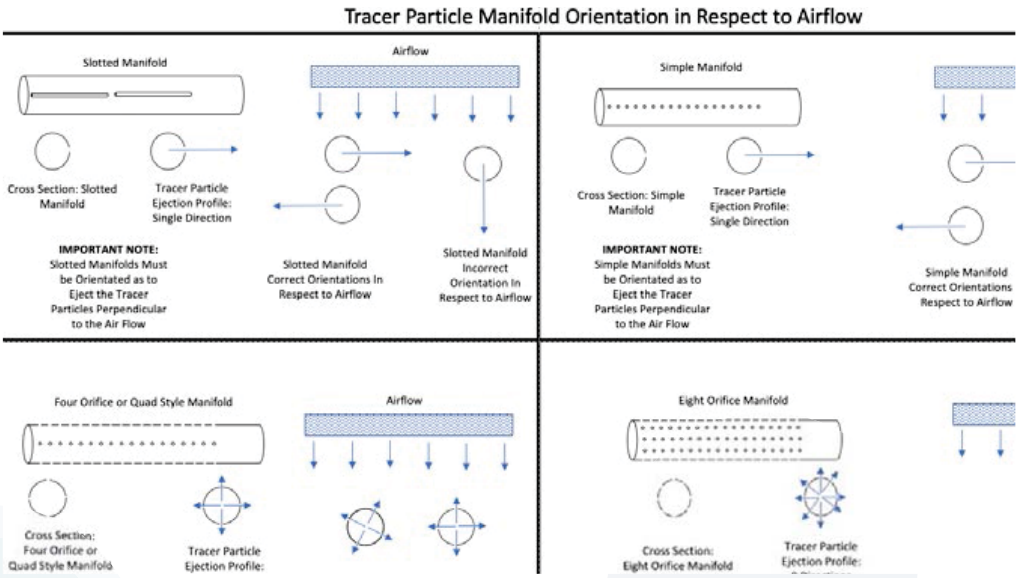

Figure 7: Tracer Particle Manifold Orientation in Respect to Airflow

STANDARDS, REGULATIONS AND GUIDLINES

ISO Technical Committee 209 has published various standards related to cleanrooms. Important concepts such as cleanroom principals, design, testing, start-up, qualification, operation, and monitoring are addressed in these standards. Designated "Cleanrooms and Associated Controlled Environments" ISO 14644 parts 1-16. Of these standards, ISO 14644 Parts 1-3 apply to all cleanrooms and controlled environments regardless of industry or function. Although these standards are not industry specific, they provide the basis for discussions related to cleanroom qualification, testing and operations. In addition, the FDA, EMA, PIC/S, USP and CETA have commented on the use of airflow visualization.

International Cleanroom Standard ISO 14644-3:2019

The ISO 14644 series of standards provide the fundamental information related to cleanrooms and controlled environments. "ISO 14644-3:2019 Part 3 Test Methods" provides guidance on test methods, recommended test apparatus and procedures to support testing and qualification of cleanrooms and controlled environments. Guidance for airflow visualization testing referred to as "Airflow direction test and visualization" is addressed in this standard as a means to demonstrate airflow direction and uniformity of velocity for comparison to the design and performance specifications for the cleanroom or clean zone being tested.

The standard introduces the Tracer Particle Injection method where the test is carried out by the observation and recording the behavior of Tracer Particles. In addition, the standard introduces the apparatus (Tracer Particle Generator) and methods from which the tracer particles may be generated. Additional caution is provided related to the tracer particle size and the effects of gravity. Tracer particles should of a suitable size to be observed and recorded, but not so large that gravity or other effects will result in their behavior diverging from that of the airflow patterns.

FDA regulations and guidance
The US FDA requires and reviews airflow visualization studies for cleanrooms and critical areas. Examples of the FDA's scrutiny related to smoke studies is found in warning letters and inspectors' 483 observations. From a US Regulatory perspective, perhaps the most important guidance on Smoke Studies (Air Pattern Analysis) is found in "Guidance for Industry Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice" 2004: The expectation is the studies are meant to demonstrate and document that HEPA-filtered air is supplied at a velocity sufficient to sweep particles away from the critical area and maintain unidirectional airflow during operations. As airflow patterns are influenced by air movement, air velocity measurements should correlate with smoke studies. The goal is to document and demonstrate (video recording) that the installation can maintain unidirectional airflow and air quality under all operational conditions. "After the relevant parameters are established, it is crucial that airflow patterns be evaluated for turbulence or eddy currents that can act as a channel or reservoir for air contaminants (e.g., from an adjoining lower classified area). In situ air pattern analysis should be conducted at the critical area to demonstrate unidirectional airflow and sweeping action over and away from the product under dynamic conditions."

The studies should be well documented with written conclusions and include evaluation of the impact of aseptic interventions and equipment design. Inspectors commonly review videos of airflow visualization studies and expect that these studies are repeated at a defined frequency as that even successfully qualified systems can be compromised by poor operational, maintenance, or personnel practices.

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Clean Area Separation

Airflow Visualization Studies should include the testing of cleanroom and barrier system doors (including pass-throughs) to document proper airflow from areas of higher cleanliness to adjacent less clean areas. It is vital for rooms of higher air cleanliness to have a substantial positive pressure differential and airflow relative to adjacent rooms of lower air cleanliness (with doors closed). Airflow Visualization Studies should document and demonstrate when doors are open, outward airflow should be sufficient to minimize ingress of contamination. This information can be used in risk analysis, environmental monitoring site selection and to determine the time a door can remain ajar before alarming.

GMP Annex 1:

The 2022 updated of GMP annex 1 expands regulatory scrutiny of airflow visualization studies and addresses gaps related to this testing.

- Airflow patterns within cleanrooms and clean zones should be visualized.
- Airflow Visualization should be performed in the at-rest (static) condition and in operational (dynamic) condition while simulating operations.
- Airflow Visualization should demonstrate the absence of ingress from lower grade areas to higher grade areas via doors, mouseholes, isolator and RABS openings.
- Airflow Visualization should demonstrate 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, airflow visualisation studies should be performed in cleanrooms, RABS and isolators.

USP 797 USP General Chapter <797> Pharmaceutical Compounding Sterile Preparations

For sterile compounding facilities USP 797 provides additional requirements and guidance for airflow visualization studies, referred to in the regulation as smoke pattern testing.

- Dynamic airflow smoke pattern test: A Primary Engineering Control (RABS, Isolator, BSC, Flow Bench) test in using a visible source of smoke, which is neutrally buoyant, is used to observe air patterns within the unidirectional space under dynamic conditions. Operating personnel are present and simulating or performing compounding. The conditions should reflect the largest number of personnel and highest complexity of compounding expected during routine operations as determined by the designated person(s). This test is not appropriate for ISO Class 7 or ISO Class 8 cleanrooms that do not have unidirectional airflow (see Visual smoke study).
- Visual smoke study: A test, used in ISO Class 7 and ISO Class 8 rooms that do not have unidirectional airflow, in which a visible source of smoke, which is neutrally buoyant, is used to verify an absence of stagnant airflow where particulates can accumulate. This test does not need to be performed under dynamic operating conditions and is not appropriate for PECs (see Dynamic airflow smoke pattern test).

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Controlled Environment Testing Association (CETA) Guidance

The Controlled Environment Testing Association (CETA) made up of experienced cleanroom testing professionals in the United States, provides important considerations and procedures related to airflow visualization testing for sterile compounding facilities.

"CETA Application Guide CAG-014 Airflow Visualization Study Revised March 2022". Important points made in this guide:

- The visible medium (tracer particle) should be as close to neutrally buoyant as possible.
- Water based generators such as ultrasonic and water, CO2 and liquid nitrogen create a fog that is heavier than air and do not always provide for an accurate representation of the actual air patterns. These smoke sources also diffuse rapidly in ambient air, making identification of stagnant air, or delayed re-entrainment to the critical zones difficult to accurately identify.

USP 1116: USP General Information <USP 1116> Microbial Control and Monitoring of Aseptic Processing Environments

Though not considered compulsory, USP 1116 provides important noteworthy guidance; "Studying airflow visually is probably more useful than measuring air velocity or air exchange rates. Additionally, USP 1116 introduces the L-R (Ljundqvist & Reinmüller) method using a tracer particle generator for cleanroom airflow visualization to identify any vortices or turbulent zones. This can be done to fine tune cleanroom airflow and for risk assessment. After the visual optimization of cleanroom airflow, smoke studies are done in the critical zone during simulation of production conditions with equipment and personnel in place. By using a tracer particle generator and an airborne particle counter a physical risk assessment can be conducted to evaluate the ability of RABS and Isolator system to resist contamination.

COMMON MISTAKES AND MISUNDERSTANDINGS RELATED TO AVS

Because the performance of airflow visualization studies is not universally understood, the following is a list of common mistakes associated with airflow visualization studies.

1. Smoke Studies were not comprehensive and did not include simulations of all activities such as automatic filling and closing operations, corrective & inherent interventions (including set-up and environmental monitoring).
2. Smoke Studies did not have the correct number of people per media fills.
3. Smoke Studies were not used as part of the risk assessment or operator training.
4. Smoke Studies not used for establishing CCPs for Environmental Monitoring.
5. Video documentation omitted important information, location, time, and date.
6. Smoke was too dense or not dense enough.
7. The camera was obstructed by too dense of smoke or the operator.
8. The AVS Protocol, Procedure, or Training was either incomplete or missing.
9. AVS Video shows GMP violations (dirt, residue, rust, water damage...).
10. AVS Video shows poor aseptic behavior.
11. There was no test report, or the report was missing conclusions.
12. QA and Validation did not participate in the smoke study.
13. The smoke injection method influences the local air patterns (the jetting effect)
14. AVS test results show smoke refluxing back into critical areas that were not noted on the test report.
15. AVS test conditions are deliberately altered to present more favorable results. (Knowingly using fog that isn't neutrally buoyant, injecting particles with high velocity, altering manifold positions, turning of HEPAs to obscure actual air patterns and obtain more favorable results)
16. AVS videos are not retained or are not available for review.
17. AVS Videos are retained on outdated format: CD, DVD, VHS...
18. AVS Videos are in low resolution (e-mail purposes).
19. Unedited (raw) Videos are not retained, suitably stored, indexed or available.
20. Untrained personnel are utilized for simulating aseptic interventions.
21. Smoke (tracer particles) are ejected into the air stream, overpowering the local air patterns.
22. Smoke Studies were conducted using heavier than air fogging systems which do not faithfully (accurately) follow the actual airflow patterns.

- Smoke Studies did not Identify Dead Spaces
- Smoke Studies did not identify Vortices (Eddy Currents)
- Smoke Studies did not identify upwards movement of air

ADDITIONAL CONSIDERATIONS FOR AIRFLOW VISUALIZATION

A variety of factors can impact the quality of the airflow visualization testing, and documentation. Factors such as camera angles, video storage, smoke density and method of delivery can all influence the results of what is recorded.

Preparation for Airflow Visualization

Well executed and documented airflow visualization studies require preparation. A clear understanding of what areas are to be tested, what activities are to be simulated, the testing methodology to be used. Factors such as acceptance criteria and the recording methods should be defined before the testing commences. Airflow visualization studies that are intended for regulatory or customer review should utilize a protocol that addresses these considerations. If the studies are for engineering purposes or investigative in nature, a less formal approach can be taken.

- In preparation for the studies, make sure all cleanrooms adjacent to the area being tested and on the same air handling system are not in use.
- The cleanrooms and clean zones including RABS, Flow benches BSCs and Isolators should already be qualified and operational.
- Gowning requirements for test personnel need not be the same as production operators as the areas being tested will be extensively cleaned after the smoke study.

- Smoke detection systems should be in stand-by mode and local fire authorities alerted prior to conducting smoke studies.

Cameras

Suitably placed video cameras are critical to AVS. The correct camera angle, focus and recording method play an important part in the clarity of the smoke study video and can influence the conclusions related to the airflow studies. Technological advances in camera technology and data storage have enhanced AVS. There are a wide variety of camera options available. The small size and portability of smart phones and sport cameras allow for cameras to be placed inside critical zones, allowing for more comprehensive understanding of air patterns.

Time and Date

An important consideration for AVS is documenting and recording the study. It is important the video start with a marquee or sign displaying testing information, so the viewer is introduced to the test and is aware of testing details. Important information on this marquee or sign is the time and date of the AVS. The internal clock of any computer, camera, smart phone or sport camera used for the AVS, should be synced to the local time and date. This is important as original unedited video files will have metainformation including the timestamp of when the video was recorded. If the clock of the camera is not correct, the timestamp of the video will not match the documentation. In order to avoid any confusion in the audit trail, make sure all relevant clocks are synced. It is not necessary to have the camera automatically overlay the time stamp (camcorder effect) on the recorded raw video as this information is retained in the original video's metainformation.

The use of multiple cameras or camera networks

The use of multiple cameras allows the recording multiple angles of the same test. This is helpful as sometimes in tight spaces, operator movements may obstruct one camera, having additional cameras at alternate angles and locations allows the documentation of the air patterns to continue. The use of multiple cameras may require the use of editing software to splice the videos from the different cameras into a more manageable and viewable single summary or report video. The original videos remain unaltered and available for review or comparison.

VIDEO STORAGE AND INDEXING

For videos that will be available for regulatory scrutiny, it is imperative that all videos be suitably indexed and available for review. It is not necessary or advisable to utilize the original camera media card as the primary storage. A secure location on a network server or a dedicated hard drive suitably indexed, labeled, and backed up are commonly acceptable methods. Older technology such as video tape, DVD, CD are not widely used, making review difficult.

DOCUMENTATION WITH CONCLUSIONS

Appropriately trained airflow visualization test personnel and end user representatives should document the airflow tests with conclusions on a test report. The conclusions should be made at the time of the testing. It is important to consider that first-hand experience provides much more detail than anything captured on video. If adverse air is observed, it may be necessary to repeat the testing to best document the phenomena for management or engineering.

POST AIRFLOW VISUALIZATION STUDY CLEANING

There is nothing clean about a “Smoke Study”. Because the number of tracer particles required for suitable testing exceeds the cleanroom’s particle concentration limits, cleaning the cleanroom after the smoke study is required. Additionally, the type of Airflow Visualization that is required by the FDA and other international regulatory bodies requires smoke studies to be performed in conjunction with the simulation of processing tasks, filling and closing of containers, loading, and unloading of freeze dryers, inherent and corrective interventions including aseptic connections. As these simulations mimic actual operations with additional testing personnel, equipment (cameras, Tracer Particle Generator, tripods etc.) inside or in close proximity of the critical area, a deep cleaning inclusive of sanitization, disinfection and sterilization must be undertaken.

REFERENCES

- ISO 14644-3: 2019 Cleanrooms and Associated Controlled Environments: Part 3 Test Methods.
- Guidance for Industry Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice” 2004. US FDA
- 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: Annex 1 Manufacture of Sterile Medicinal Products
- USP General Chapter <797> Pharmaceutical Compounding Sterile Preparations
- USP General Information <USP 1116> Microbial Control and Monitoring of Aseptic Processing Environments.
- CETA Application Guide CAG-014 Airflow Visualization Study Revised March 2022