

Characterization of Airflow Patterns, Identification of Barrier System Design Flaws, and Cleanroom/Barrier System Integration Mistakes

Characterization of Airflow Patterns, Identification of Barrier System Design Flaws, and Cleanroom/Barrier System Integration Mistakes

Morgan Polen
Contamination Control Expert
Microrite, inc.

Aerospace, Defense, Electronics, Automobile, & Energy Industries Use Air Flow Visualization

Understanding the effects of air flow over objects such as airplane wings or automobiles, is used to evaluate designs.

It is extremely important that the visualization cloud (made up of Tracer Particles), **faithfully follow the streamlines of the air flow**, allowing the analysis and evaluation of the physical (Actual) air flow patterns against design and operational requirements.



Appropriately controlled, filtered and directed air provides the “Contamination Control Effect” essential to cleanroom operations.

Analysis of airflow patterns is required by regulatory bodies as part of the qualification of aseptic or sterile manufacturing operations.

Additionally air pattern analysis is a very useful tool in contamination control for all cleanroom classes.

This valuable tool is:

- Misunderstood: (Numerous Warning Letters and 483 Observations)
- Often Underutilized (Only performed once at qualification and repeated ~2 years)
- Easily Falsified
- Often incorrectly Applied

Smoke Studies are Often Approached as a Rubber Stamp Test

Often Conducted Under the Assumption That They Will Pass Because Other Cleanroom/Barrier System Tests Have Passed:

- ✓ Air Volume and Air Velocity
- ✓ Differential Pressure
- ✓ Filter Integrity
- ✓ Particle Count



Heavier Than Air Fogging Systems for Air Flow Visualization (Smoke Studies) do not Faithfully (accurately) follow the Actual Air Flow Patterns.

- ✓ Do not Identify Dead Spaces
- ✓ Do not identify Eddy Currents

Water, CO₂, Nitrogen Foggers are Unsuitable

Resulting in the acceptance of unsuitable air patterns

Aseptic operations being carried out in turbulent or deviant air flow conditions

Unsuitably Qualified Cleanroom/Barrier Systems and Manufacturing Operations

The results of aseptic operations being carried out in turbulent or deviant air flow conditions:

- Patient Harm from Contaminated Products
- Failed Media Fills
- Rejected and Scraped Products
- Inspectors Comments and warning letters related to “Smoke Studies”
- Inspectors Comments and warning letters related to “Data Integrity”
 - Due to the falsification of EM data or Media Fill Results
(Because the Cleanroom/Barrier System is Poorly Designed/Integrated)
- Product Import Notices/Loss of Customers

Data Integrity issues have been traced to problematic smoke studies that incorrectly qualify cleanroom/barrier systems for aseptic operations.

Blatantly obvious design flaws can be masked or hidden in smoke studies via:

- The smoke study technique.
- Tracer Particle selection or method (the use of water, CO₂ or Nitrogen fog can mask dead spaces, eddy currents or excessive turbulence)
- Camera angles.
- Documentation.

What Companies Do After a Bad Smoke Study (Air Flow Visualization):

- A. Modify cleanroom/barrier system or personnel behavior to address the problem and repeat the testing.
- B. Increase the EM for the location /operation, include this in the risk analysis.
- C. Argue about “Subjective Nature of the Study” and continue operations.
- D. Ignore the results, find another testing agency and hope they don’t find the problems.
- E. Repeat the study and manipulate the test in order to get a more favorable result.

- Air flow visualization is the science of making air flow patterns visible.
- Because air is transparent, air flow 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. The resulting cloud of tracer particles makes the air patterns visible.
- It is important that the tracer particles faithfully follow the streamlines of the air flow, allowing the analysis and evaluation of the physical (Actual) air flow patterns against design and operational requirements.
- (The term “Smoke Study” is often used synonymously for air flow visualization as Initially these studies utilized smoke sticks, cigarettes ect...)

- Understanding airflow patterns in cleanrooms and controlled environments is an extremely important aspect of cleanroom contamination control. The importance of these studies in aseptic operations cannot be overstated.
- The actual (or physical) Contamination Control Effect of cleanroom air flow can only be effectively understood when it is visually represented.
- Next to Media Fills the characterization of air patterns provides the most comprehensive over-all representation of a facilities contamination control strategy by providing:
 - A visual representation of the contamination control effect of air flow patterns in the cleanroom, barrier systems and the interface between areas of different classification/grades e.g., Grade A RABS with a GRADE B background.
 - A visual representation of operator movements, positions and attitudes in respect to maintaining the air flow's contamination control effect during simulations of operations.

Poorly conducted Air Flow Visualization Studies have resulted in the Qualification of Cleanroom/Barrier systems that are **Unsuitable for Aseptic and Low Bioburden manufacturing.**

[Play Video](#)





NOTE:
Ceiling has delamination of material. Filter has excessive Sealant at the edges. Multiple Regulator Inspection Reports, indicate Leaks were observed throughout the facility.

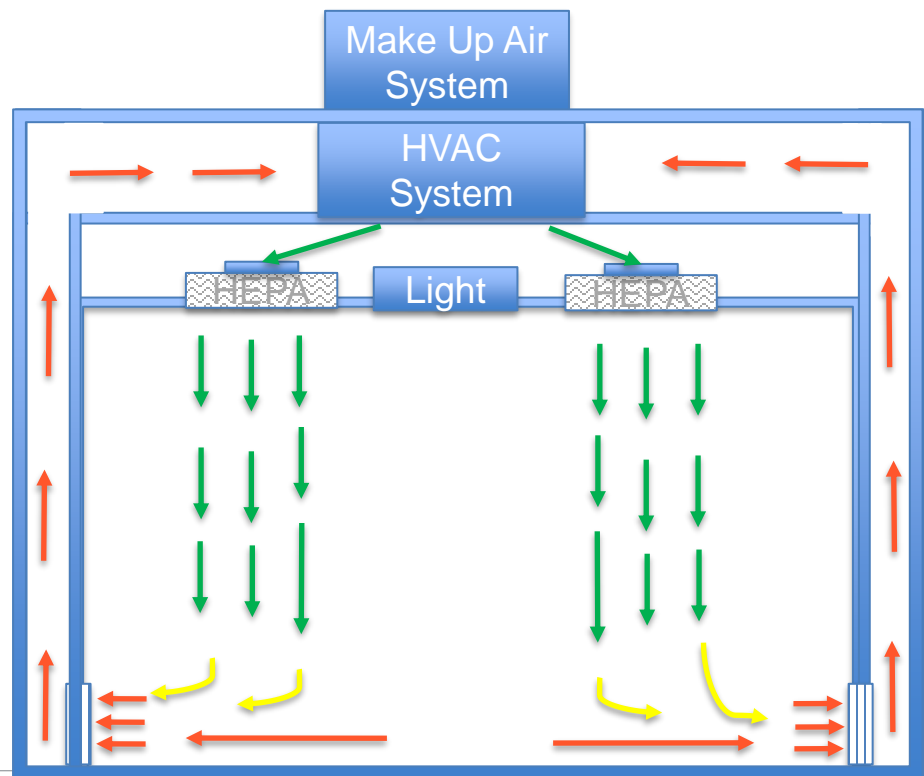
Smoke Study Videos: More than Just Watching Smoke Move Through a Facility

Customer’s comment ”The torn diffuser DID NOT impact Environmental Monitoring, so it was not required to be changed prior to the Smoke Study or the last 6 months of Aseptic Production.

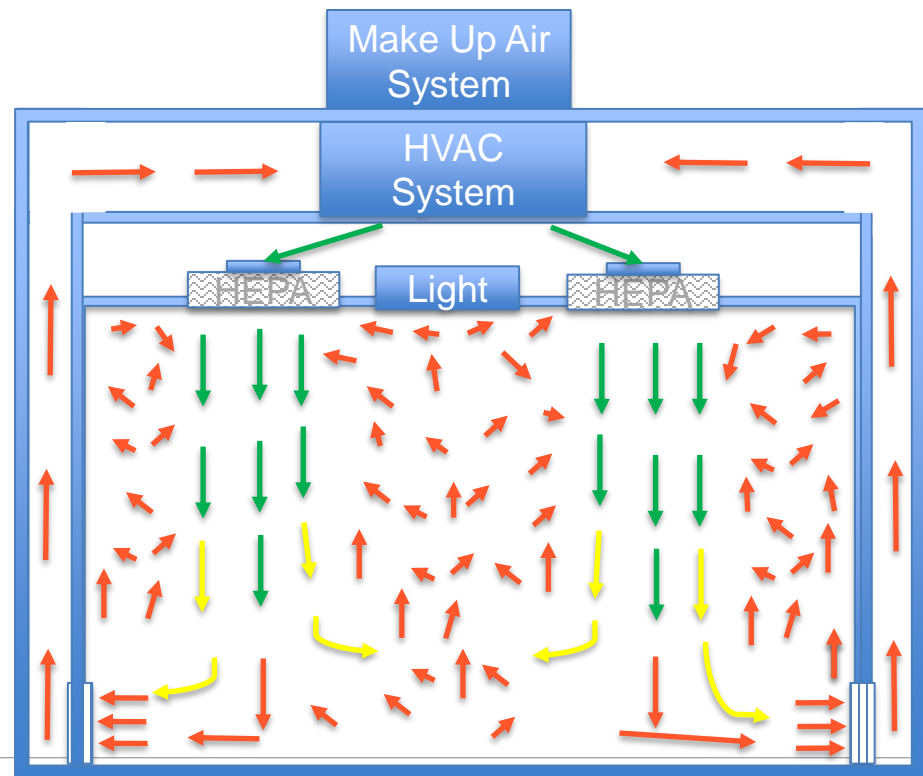


- Smoke Studies when performed correctly can:
 - Identify air flows that act as a transport channel for contamination from sources such as people, equipment or less clean areas.
 - Identify air flows that are swirling air (eddy currents) that can act as a reservoir for contamination.
 - Identify absent air flow (dead spaces).
 - Identify un  [Play Video](#)  n across the cleanroom
 - Identify errors in facilities, such as turned off LAFs and HEPA filter modules, blocked or misbalanced air returns.
 - Be a useful tool as part of a risk assessment for choosing CCPs for continuous or routine monitoring as part of a contamination control policy.

Perceived Air Flow



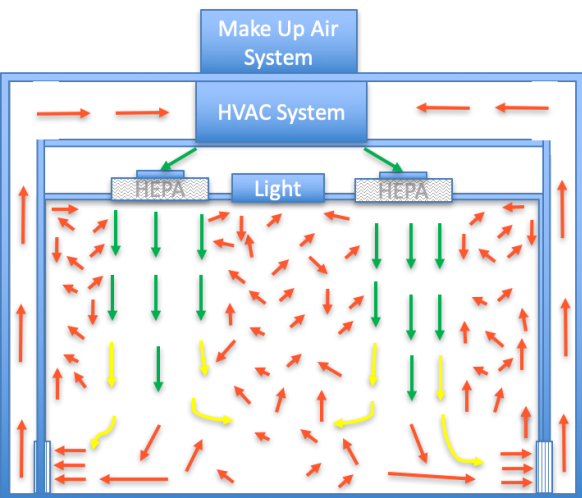
Actual Air Flow



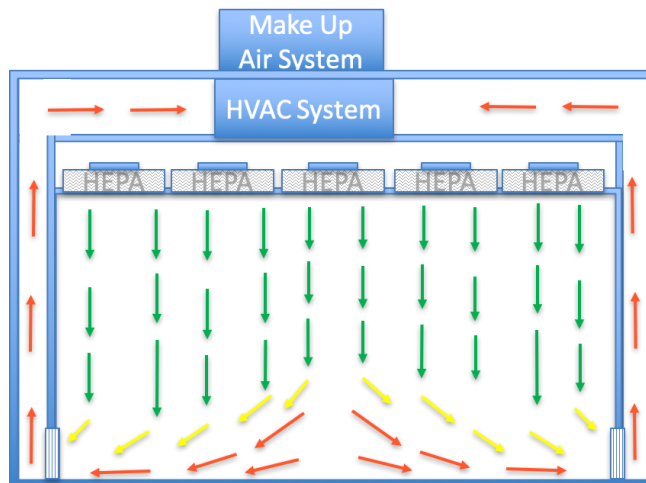
Air Flow Visualization is a Tool That is Useful for All Types of Cleanrooms

Understanding the Contamination Control Effect of Non-unidirectional Flow and Combination Flow Cleanrooms is Extremely Useful in Assessing Risk of Contamination

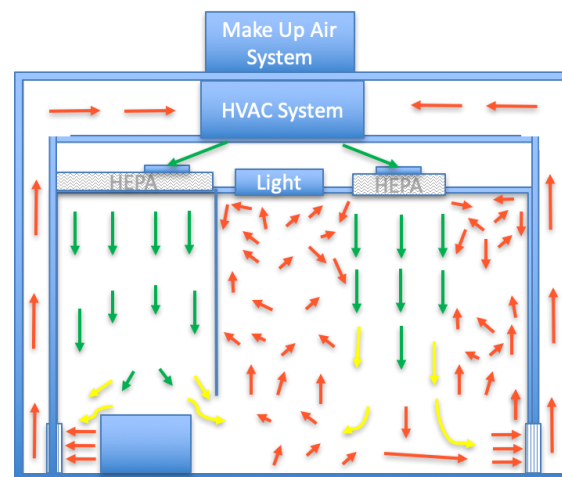
Non-Unidirectional Flow Cleanroom Concept



Unidirectional Flow Cleanroom Concept



Combination Flow Cleanroom Concept



Case History #4: RABS Inlet Overpowers Air Returns

Poor Cleanroom Design, Poor RABS Design, Poor Integration, Poor Qualification and Poor Operation:

[Play Video](#)

Partially Blocked Air Return Duct, Cleanroom Wipers, Autoclave Bags and Settle Plates Were Found.

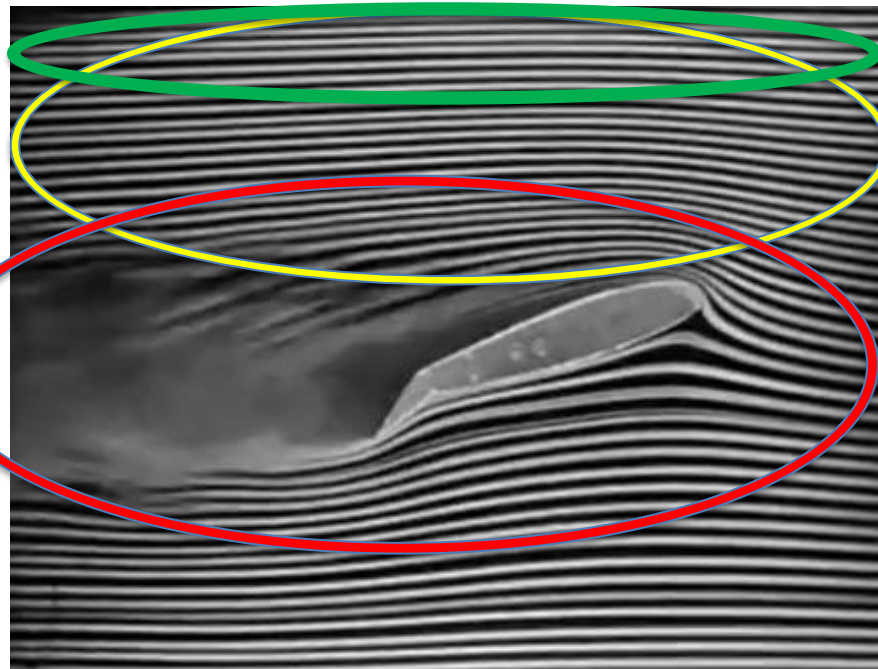
Laminar flow:

“In fluid dynamics, laminar flow (or streamline flow) occurs when a fluid flows in parallel layers, with no disruption between the layers. At low velocities, the fluid tends to flow without lateral mixing, and adjacent layers slide past one another like playing cards.

There are no cross-currents perpendicular to the direction of flow, nor eddies or swirls of fluids. In laminar flow, the motion of the particles of the fluid is very orderly with all particles moving in straight lines parallel to the pipe or containment walls.”

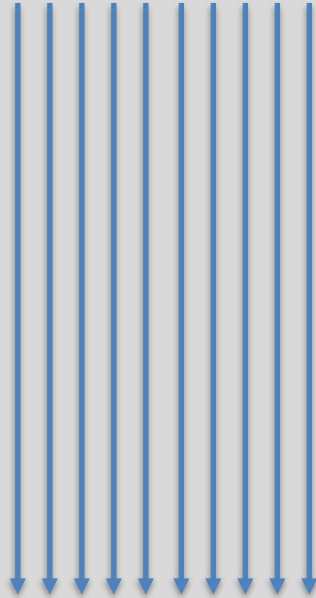
Unidirectional flow: **Laminar flow:**

Turbulent flow:



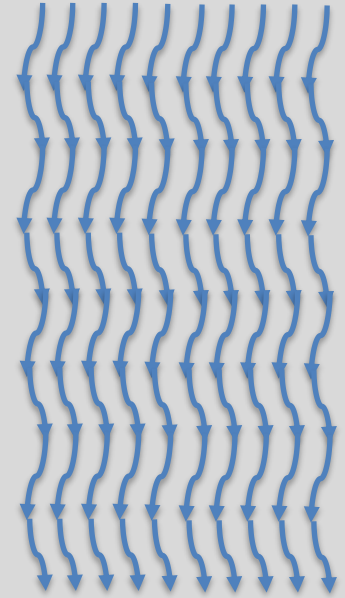
Laminar Air Flow as Defined in Draft GMP Annex 1:
“An airflow moving in a single direction and in parallel layers at constant velocity from the beginning to the end of **a straight line vector.**”

Laminar Air Flow or
streamline flow,
No Lateral movement



Unidirectional Air Flow as Defined in Draft GMP Annex 1:
“An airflow moving in a single direction, in a robust and uniform manner, and at sufficient speed to reproducibly sweep particles away from the critical processing or testing area.”

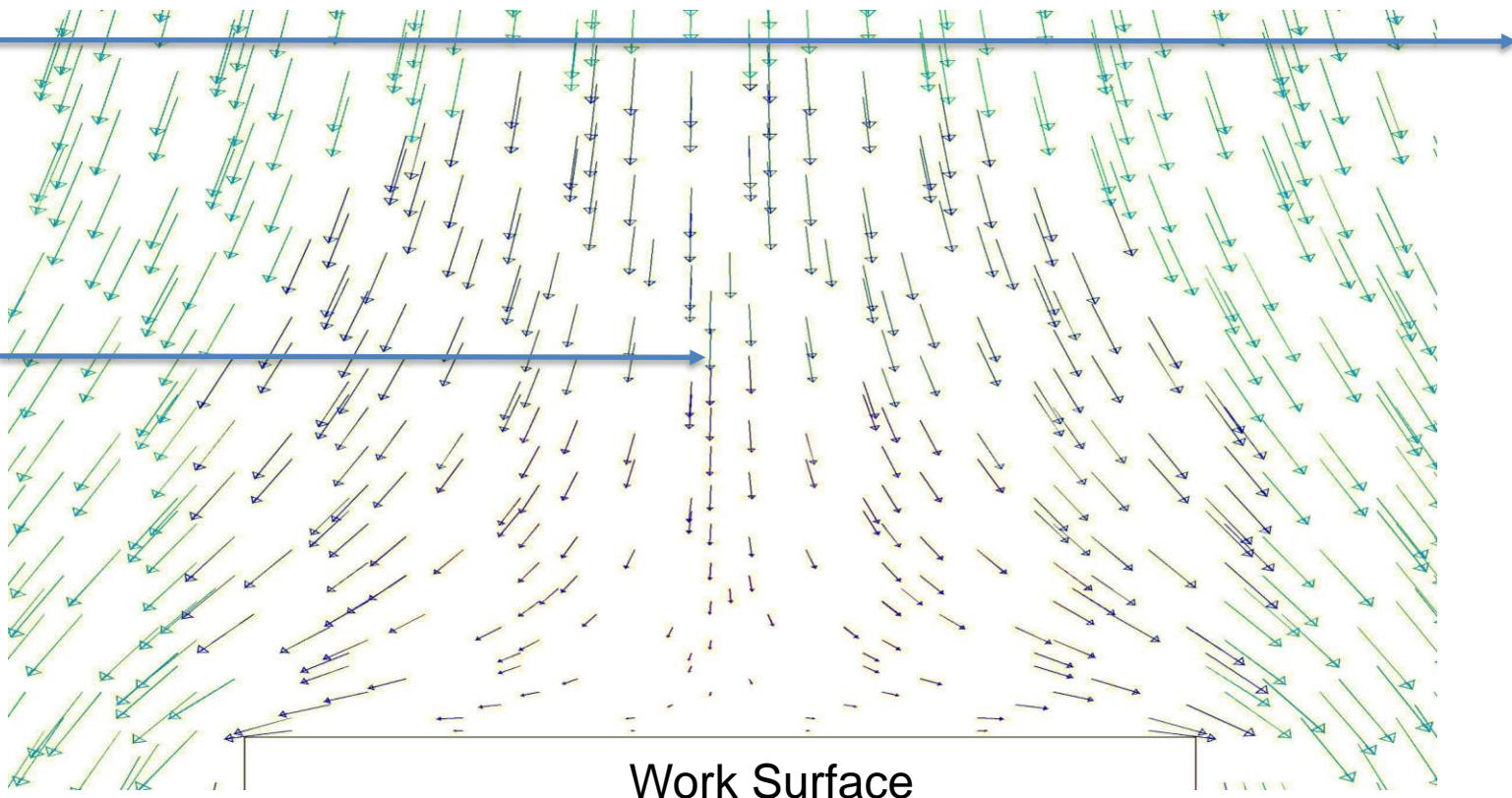
Typical Unidirectional
Cleanroom Air Flow @
90ft/min (.46m/sec)
with lateral movement



15cm (6in)
from Diffuser

What Point is
Considered
The Exit
Plane?

Air Velocities
and Direction
are not
Maintained



Work Surface

Aside from the regulatory requirements, Air flow visualization is a useful tool for assessing risk of contamination from personnel and processing.

Personnel, equipment and material flow can influence airflow and affect contamination levels in the most well designed and integrated cleanrooms and barrier systems.

Often Air Flow Visualization studies are only performed once at the initial qualification only with the intent of meeting a regulatory requirement. However, this limited approach does not take advantage of these studies as a contamination control tool that can help reduce the risk of contamination to products and product contact surfaces.

Air Flow Visualization studies when used as part of a holistic and comprehensive strategy can assist in reducing the risk of contamination by assessing the physical Contamination Control Effect of air flow when utilized in;

- a) Cleanroom Qualification for both unidirectional and non-unidirectional flow cleanrooms
- b) Barrier System FAT/SAT Qualification.
- c) Pass-through and Air Lock Qualification.
- d) Optimization of cleanroom and barrier systems integration.
- e) Optimization of operator movements, standing positions, equipment and material handling.
- f) Selection of Environmental Monitoring locations.
- g) Troubleshooting contamination issues and finding sources of air patterns that may act as a channel or reservoir for contamination.
- h) Providing a training resource for operating personnel as a pro-active approach to contamination control.

Air flow visualization testing is addressed in the international cleanroom standard. **ISO 14644-3:2019 Part 3 Test Methods**

4.2.3 Airflow direction test and visualization:

The purpose of this test is to confirm either the airflow direction or airflow pattern or both in regard to the design and performance specifications.

B.3.2

- a) Tracer thread method;
- b) Tracer injection method;
- c) airflow visualization method by image processing techniques;
- d) airflow visualization method by the measurement of velocity distribution.

By methods a) and b), airflow in the cleanroom or clean zone is actually visualized by the use of fiber tracer thread, or tracer particles. Devices, such as video cameras, record the profiles. The fiber tracer thread or tracer particles should not be a source of contamination and should follow the airflow profile accurately. Other apparatus such as a tracer particle generator, and high intensity light source may be used for these methods.

Air flow visualization testing is addressed in the international cleanroom standard.

ISO 14644-3:2019 Part 3 Test Methods

B.3.3.2 Tracer injection method

The test is carried out by observation or imaging of the behavior of tracer particles, which can be illuminated by high-intensity light sources. The test provides information about the airflow direction and uniformity of velocity in a cleanroom, clean zone or controlled zone.

The tracer particles can be generated from materials such as de-ionized (DI) water, sprayed or chemically generated alcohol/glycol, etc.

The source should be carefully selected to avoid contamination of surfaces.

The desired size of droplets should be considered when selecting the droplet generation method.

Droplets should be large enough to be detected with the available image processing techniques, **but not so large that gravitational or other effects result in their motion diverging from that of the airflow being observed.**

What are the Regulatory Requirements for Air Flow Visualization?

Cleanrooms for sterile or aseptic processing require additional design, qualification and operational considerations than are detailed in the ISO 14644 series of standards.

The US FDA “Guidance for Industry Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice” 2004:

“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 manipulations (e.g., interventions) and equipment design. Videotape or other recording mechanisms have been found to be useful aides in assessing airflow initially as well as facilitating evaluation of subsequent equipment configuration changes. It is important to note that even successfully qualified systems can be compromised by poor operational, maintenance, or personnel practices.”

“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.”

European Commission Eudralux Volume 4, Annex 1 & PIC/S GMP Guide (PE 009-11) Annex 1 Clause; 3,53,54:

- 3, “The maintenance of laminarity should be demonstrated and validated.”
- 53, “A filtered air supply **should maintain a positive pressure and an air flow relative to surrounding areas of a lower grade under all operational conditions and should flush the area effectively.**”
- 54, “It **should be demonstrated that air-flow patterns do not present a contamination risk**, e.g. care should be taken to ensure that air flows do not distribute particles from a particle generating person, operation or machine to a zone of higher product risk

Additional Regulatory Guidance for Air Flow Visualization?

USP Section 1116: Microbiological Control and Monitoring of Aseptic Processing Environments, “The maintenance of laminarity should be demonstrated and validated.”

In the evaluation of air movement within a clean room, studying airflow visually by smoke studies or other suitable means is probably more useful than using absolute measures of air-flow velocity and change rates.

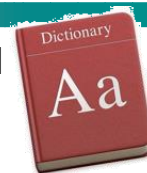
Physical Evaluation of Contamination Control Effectiveness: Following visual optimization of airflow, particulate matter is generated close to the critical zone and sterile field. This evaluation is done under simulated production conditions but with equipment and personnel in place. Known as the **L-R method**, this method challenges the entire system’s air ventilation system by using a tracer particle generator in conjunction with a particle counter.

This allows analysts to visualize the air movements throughout a cleanroom or a controlled environment, including vortices or turbulent zones, and the airflow pattern can be fine-tuned to minimize these undesirable effects.

This type of test can also be used to evaluate the ability of RABS and isolator systems, particularly around product exit ports in these systems, to resist the effects of contamination.

What is In Situ Air Pattern Analysis?

US FDA “Guidance for Industry: “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.”



in situ / Latin (In 'sɪtju:.) / adverb, adjective (postpositive) in the natural, original, or appropriate position situated in the original, natural, or existing place or position



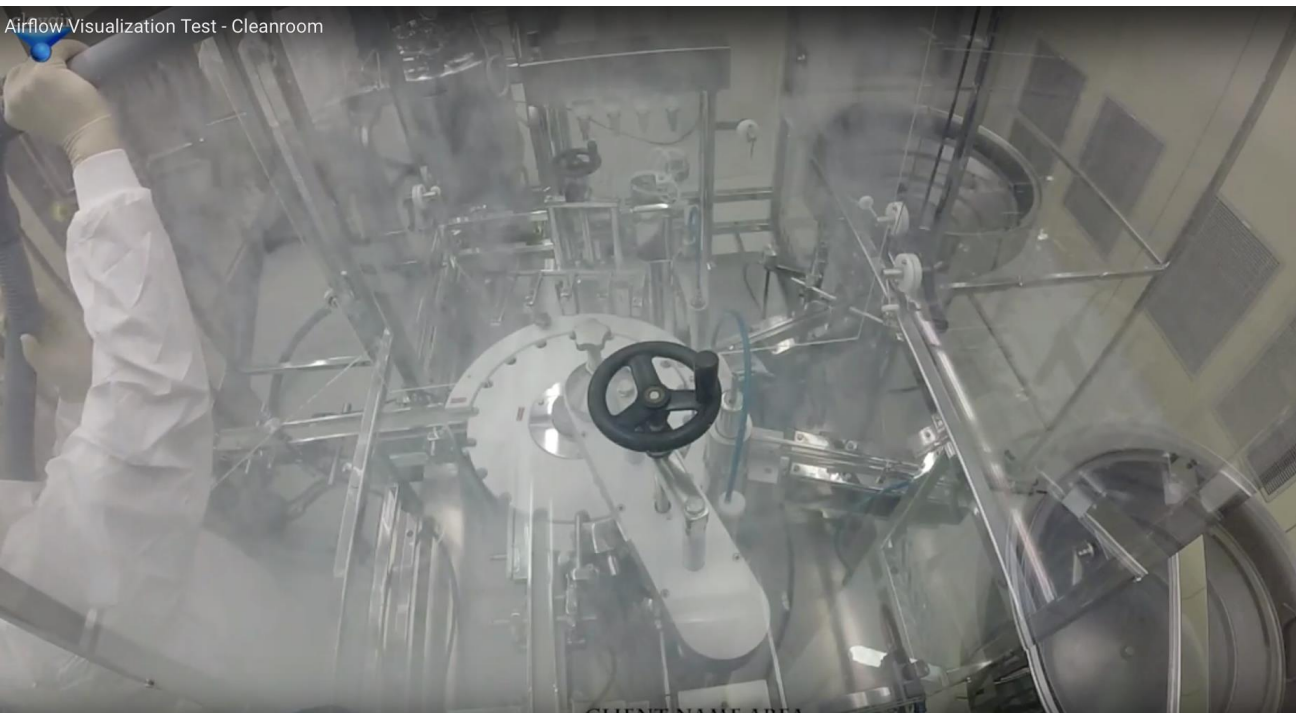
How can you Accurately Evaluate Air Flow Patterns while Simulating Operator Movements with an Extra Person Holding a Smoke Wand?





in situ / Latin (in 'sɪtju:) / adverb, adjective (postpositive) in the natural, original, or appropriate position situated in the original, natural, or existing place or position

Airflow Visualization Test - Cleanroom



How can you Evaluate a Barrier System's Air Flow Patterns with the Door Open?

- FDA “cGMP Regulations were established to be Flexible”
- "C" in cGMP stands for "current," requiring companies to use technologies and systems that are up-to-date in order to comply with the regulations.
- This is intended to allow each manufacturer to decide individually how to best implement the necessary controls by using:
 - Scientifically sound design
 - Processing methods
 - Testing procedures
- The flexibility in these regulations allows companies to use modern technologies and innovative approaches to achieve higher quality through continual improvement.
- It is important to note that cGMPs are **minimum requirements**.
- Systems and equipment that may have been "**top-of-the-line**" to prevent contamination, mix-ups, and errors **10 or 20 years ago** may be less than adequate by today's standards.

* Poor cGMP conditions at a manufacturing facility can ultimately pose a life-threatening health risk to a patient

*FDA: Guidance for Industry Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice

The Most Important Aspect of Conducting Air Flow Visualization Studies is determining the actual (physical) Contamination Control Effect of cleanroom air flow. (Too Often: Perceived air flow \neq Actual (Physical) air flow)

Because Smoke Studies are **extremely invasive in nature**, additional equipment and personnel not associated with Operations are going to be working inside Grade A (ISO 5) environments and coming in contact with Production equipment, barrier systems, Doors, Walls and floors.

Tracer Particle Concentration (for the testing) >1 million particles $\geq 0.5\mu\text{M}/\text{m}^3$

The Longer the Testing, the greater the number of particles impacted on filters.

A comprehensive decontamination of the cleanroom, barrier system, processing equipment is required. The required level of cleaning and subsequent environmental monitoring is typically addressed in basic SOPs.

Additional Guidance for Air Flow Visualization?

Controlled Environment Testing Association (CETA)

CETA Certification Guide for Sterile Compounding Facilities CAG-003-2006 -13

Revised May 20, 2015

13.3 A visible source of smoke such as a **glycol based fog generator** or ventilation smoke tube is used to observe air patterns within the unidirectional space.

Smoke is generated directly downstream of the diffuser and then observed as it flows across the critical site and the direct compounding area (DCA) and to a return or out of the critical area. Air exiting the critical area should not re-enter.

Additional Guidance for Air Flow Visualization?

CETA Certification Guide for Sterile Compounding Facilities CAG-003-2006 - 13 Revised May 20, 2015

13.5 Water based fog generators such as 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.

The **smoke source should be as close to neutrally buoyant as possible**. For example, when generating the fog in an area with no detectable airflow, it should not “fall out” or “drop”.

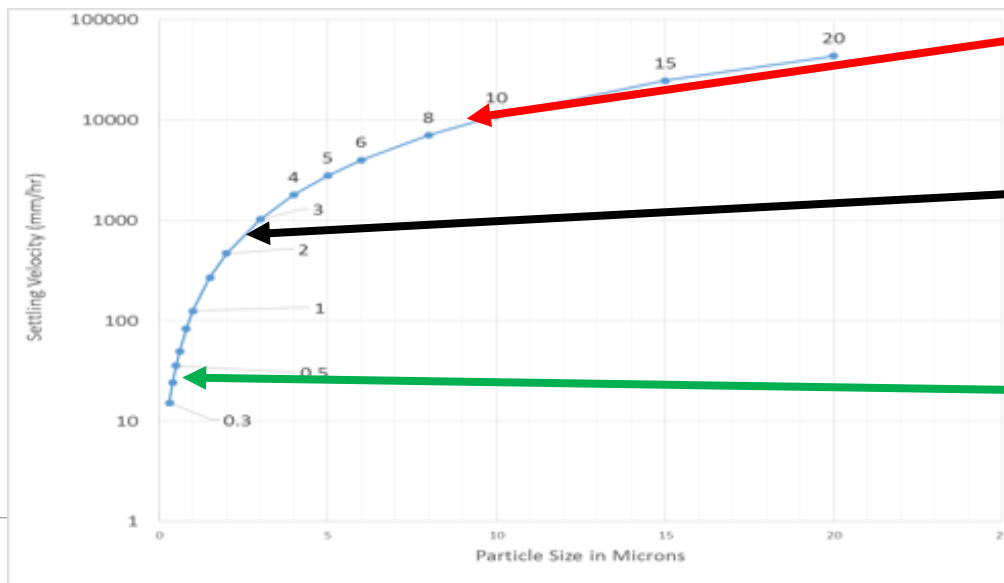
Fog streams that are heavier than air may not detect updrafts and turbulence that are detected with a generally neutral buoyant detection stream.

What is Neutral Buoyancy?

A. For Cleanroom Applications Particles with neutral buoyancy, tracer particle behavior is influenced only by air currents within the cleanroom or area being tested. Gravity and the particles temperature do not influence the particles behavior during the testing.

Chart 1: *Particle Settling Velocity

Chart 1:
Particle Size in Microns
verse Settling Velocity
mm/hour
in STILL AIR at 20 °C



Ultrasonic Tracer Particles
5µM – 10µM

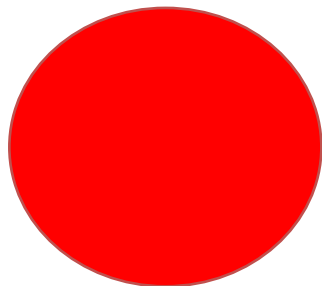
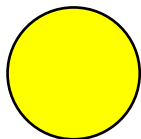
Nitrogen Tracer Particles
2µM – 5µM

Sub-micron Glycol Tracer Particles
0.3µM - 0.5µM

Nitrogen Fog
Tracer Particle
 $2\mu\text{M} - 5\mu\text{M}$

- Glycol Fog
Tracer Particle
 $0.3\mu\text{M} - 0.5\mu\text{M}$

Ultrasonic Water Fog
Tracer Particle
 $5\mu\text{M} - 10\mu\text{M}$



The Tracer Particles cloud should be visible from the source (HEPA) to the return. Any media that does not have neutral buoyancy and **cannot** be visualized from the source to the return (in a single test) following the air patterns is not suitable for air flow visualization of cleanrooms.

Such systems have created false conclusions regarding air patterns in cleanrooms and barrier systems.

These false conclusions have led to failed media fills, contaminated products, inspector's observations, warning letters and patient harm.

Brand new Aseptic Liquid Filling line (CMO)

- Failed Initial Media Fills (**Qualification Smoke Study was done with a Nitrogen Fogger**).
- Proper Smoke Study Investigation identified cleanroom and RABS were not suitable for aseptic operations.
- Project delayed while cleanroom was modified, however RABS was not modified.
- After the Cleanroom was modified Smoke Studies Identified turbulence in the RABS (stopper and crimper loading ports).
- Customer rejected the latest Smoke Study testing (showing turbulence and air flow reversal).
- Testing was repeated with a Nitrogen Fogger. This did not indicate the turbulence and created a Data Integrity Situation.





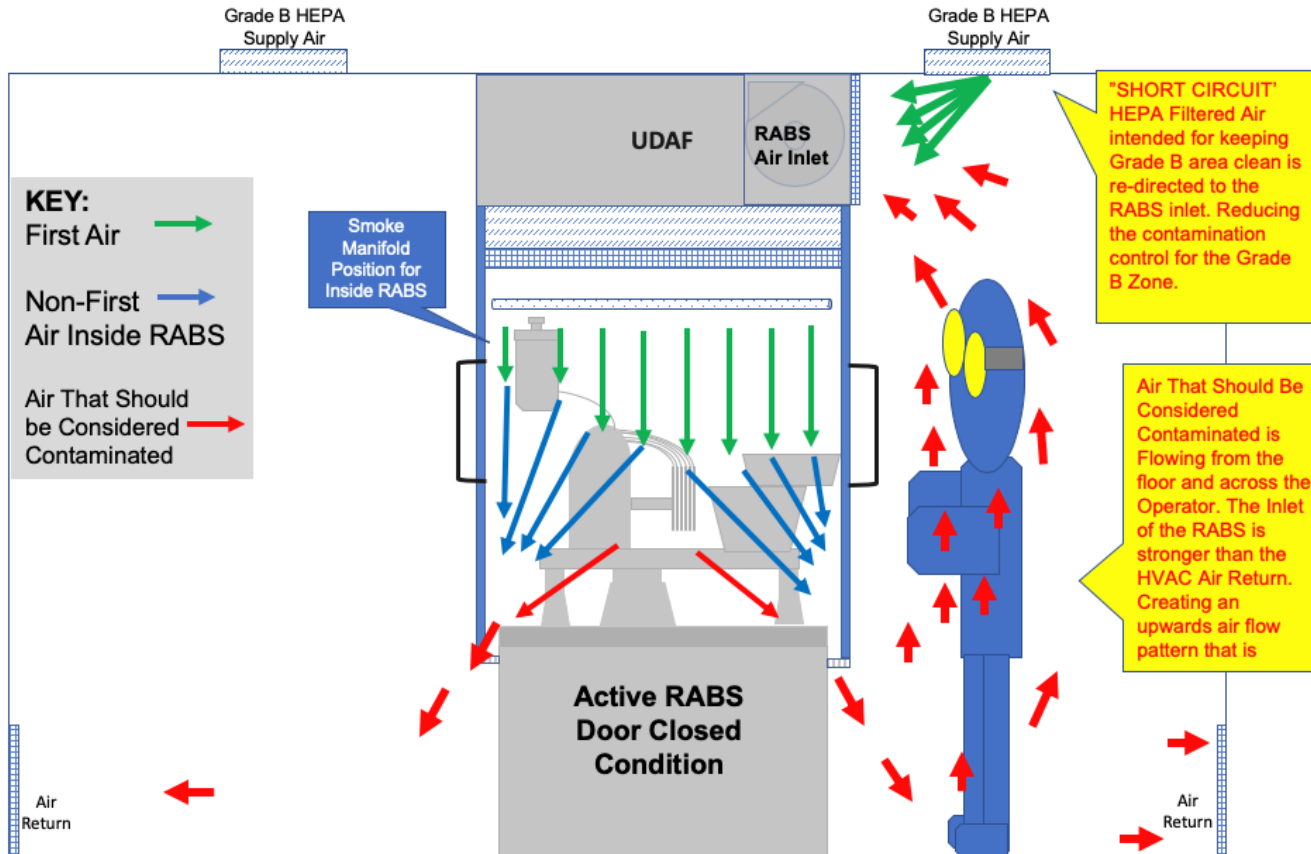
Passive RABS (decoupled from cleanroom HEPA Filters) Case History ³⁹

[Play Video](#)



Nitrogen Cleanroom Fogger verse a Glycol Based Fogger: **HEPA OFF Condition**

Play Video



Types of Smoke Studies

Investigative Air Flow Visualization Studies

(Diagnosing Contamination Problems, Early Confirmation of Design Criteria)

Engineering Air Flow Visualization Studies:

Gaining an Understanding of Air Patterns, Personnel Movement for the Purposes of Optimization and Risk Analysis, prior to Qualification

Static Air Flow Visualization Studies

Studies Conducted without Personnel Movement (A pre-cursor for Dynamic Studies)

Dynamic Air Flow Visualization Studies:

Studies Conducted with Personnel and Equipment Conducting Simulations or operations

In Situ Static/Dynamic Air Pattern Analysis

The “Big Deal” Smoke Studies for Documentary Purposes, for Final Qualification and Meeting Regulatory expectations.

CONCLUSION: Airflow Visualization as Part of a Contamination Control Strategy⁴²

The Smoke Study/AFV is used to Evaluate the Risk of contamination from the effects of the **actual air flow patterns by performing** simulations while introducing Tracer Particles during:

- Normal Operations
- Personnel interactions and movement / interventions / equipment assembly / Environmental Monitoring
- Surrounding environment

This Evaluation of the actual air flow patterns is used to:

- Identify Risk associated with the Cleanroom/Barrier System Design and Integration
- Identify Risk associated with Normal Operations, Interventions, equipment assembly and EM
- Establish Risk-Based Monitoring Locations for Viable and Non-Viable Particles

Initial Smoke Studies/AFV should be conducted as part of engineering and manufacturing studies to optimize equipment and operators movements/positions prior to qualification of the cleanroom and repeated after optimization as documented "In situ Air Pattern Analysis", prior to 1st media fills.

These studies should be used for evaluation and training as the final details of aseptic processing techniques are being worked out prior to doing the 1st medial fills

Smoke Study/AFVs are not for the evaluation of aseptic techniques, though the simulation of operations is required, close attention to detail even during the qualification stage (prior to 1st media fills) is important.

Pay attention to personnel behavior.

REMEMBER!

INSPECTORS ARE GOING TO REVIEW YOUR SMOKE STUDY VIDEOS!

Thank You For Your Attention

