APPLIED PHYSICS

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TECHNICAL WHITE PAPER

Scientific Basis and Qualification Framework for Ultrasonic Water Fog as a Functionally Neutrally Buoyant Airflow Tracer

Pharmaceutical cleanroom airflow visualization (“smoke study”) applications

Regulatory position

Micron-scale water fog generated by ultrasonic atomization can be scientifically suitable as an airflow-visualization tracer when the generated droplet size, particle response, gravitational slip, plume temperature, source momentum, and study technique are controlled and qualified. The atomization mechanism itself does not determine tracer fidelity.

For a nominal 4–8 µm water-droplet range, calculated particle response times are approximately 0.05–0.20 ms and still-air terminal settling velocities approximately 0.50–1.96 mm/s. At a 0.45 m/s unidirectional airflow reference velocity, gravitational slip is approximately 0.11–0.44% of the streamwise velocity.

Prepared by Applied Physics

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Prepared for pharmaceutical, biotechnology, medical-device, and cleanroom validation organizations evaluating ultrasonic airflow-visualization media.

Document status: Scientific and engineering position paper. This document is intended to support technical evaluation and risk-based qualification. It does not replace a facility-specific protocol, executed qualification, quality-system approval, or regulatory judgment.

1. Executive Summary

Recent FDA warning letters have intensified scrutiny of airflow-visualization studies in aseptic processing. In June and July 2026, FDA directed firms to perform thorough dynamic smoke studies using proper practices, giving “neutrally buoyant media” as an example of a practice that supports appropriate visualization of airflow. The letters do not prescribe a particular fog-generation technology, a required particle density, or a numerical droplet-size limit. They focus on whether airflow is appropriately visualized under representative dynamic conditions. [2,3]

The scientific question is therefore not whether a liquid-water droplet has the same material density as air. It does not. The relevant engineering question is whether the visible tracer follows the local air motion closely enough, over the spatial and temporal scale of the study, that the observed path represents the airflow rather than the tracer’s own inertia, gravitational settling, thermal plume, or source jet.

Peer-reviewed evidence directly supports the use of micron-scale water fog as an airflow tracer. Kennedy demonstrated that water fog produced by an ultrasonic nebulizer was suitable for airflow visualization and reproduced the airflow features shown by conventional mineral-oil smoke. [6] Lozano and co-workers built an ultrasonic gaseous-flow seeder using piezoceramic elements at 1.65 MHz; it generated 4–5 µm Sauter-mean-diameter water droplets and was successfully used to visualize a free air jet and measure its three-dimensional velocity field by stereoscopic PIV. [7] Ayati and co-workers used approximately 6 µm water droplets as gas-phase tracers, validated the technique against direct numerical simulation, and concluded from Stokes-number analysis that the droplets were sufficiently small to be treated as passive tracers. [8] More recent high-resolution airflow research has successfully used 12 µm and approximately 15 µm fog droplets, with investigators explicitly demonstrating Stokes numbers far below unity. [9,10]

Those studies are especially relevant to CRF-class systems because the nominal generated-droplet range analyzed here is 4–8 µm, overlapping the published 4–6 µm range that has already been used as a quantitative gas-flow tracer. The scientific literature therefore does not support a categorical proposition that ultrasonic atomization inherently produces an unsuitable airflow tracer.

There is, however, an important qualification condition. Auvinen and co-workers used a 1.7 MHz ultrasonic nebulizer producing 6–8 µm wet droplets and observed a negatively buoyant plume immediately downstream because evaporation cooled the carrier air. They corrected the effect thermally. [12] This is not evidence that 6–8 µm droplets are intrinsically poor tracers; it demonstrates that plume-scale thermal bias can dominate particle-scale behavior if the device and release conditions are not controlled. A defensible pharmaceutical program should therefore qualify the complete tracer system—not merely cite droplet size.

> **Bottom-line conclusion**
>
> A scientifically defensible use of the term “functionally neutrally buoyant” is: a tracer whose gravitational and inertial slip relative to the local air motion is negligible for the observation scale, and whose generation system introduces no material thermal or momentum bias into the airflow being studied.
>
> Under that definition, fresh 4–8 µm ultrasonic water fog can satisfy the physics of a passive airflow tracer. Suitability should be confirmed by documented controls for droplet size, release temperature, release momentum, fog age/concentration, and execution technique.

2. Scope, Terminology, and Claims

2.1 Scope

This paper addresses visible water fog generated by MHz-range ultrasonic atomization for qualitative airflow visualization in pharmaceutical cleanrooms, restricted-access barrier systems (RABS), isolators, biological safety cabinets, laminar-flow workstations, and comparable controlled environments. It evaluates the aerodynamic suitability of micron-scale droplets as tracers and provides a qualification framework that can be incorporated into site protocols and contamination-control strategies.

2.2 What “smoke study” means in this context

“Smoke study” is a conventional industry term for airflow visualization. The tracer need not be combustion smoke. FDA itself uses “airflow visualization (i.e., smoke studies)” when discussing aseptic processing. [2] The scientific purpose is to make invisible air motion visible so that directionality, first-air protection, turbulence, eddies, reflux, ingress, and the effects of operator interventions can be evaluated.

2.3 Strict neutral buoyancy versus tracer fidelity

In strict hydrostatics, neutral buoyancy means equal effective density between an object and the surrounding fluid. A liquid-water droplet cannot satisfy that literal definition in air because liquid water is hundreds of times denser than air. That literal definition is not the criterion used in particle image velocimetry or aerosol-flow measurement. Flow-tracer suitability is evaluated by the particle's ability to follow the instantaneous motion of the carrier gas, including response time/inertia and gravitational slip; source-induced thermal and momentum disturbances must also be excluded. This engineering approach is consistent with foundational PIV tracer-selection literature. [13]

> **Definition used in this paper**
>
> Functionally neutrally buoyant airflow tracer: a visible tracer that follows the local air streamlines with negligible particle inertia and gravitational slip over the study’s relevant time and length scales, and that is introduced without a material thermal or momentum disturbance that would create an artificial trajectory.

2.4 Claims made—and not made

- This paper supports the scientific suitability of micron-scale ultrasonic water fog for airflow visualization when the complete tracer system is qualified.
- It does not claim that water droplets have the same material density as air.
- It does not claim that every ultrasonic fogger, every hose/wand arrangement, every fog concentration, or every operating condition is automatically suitable.
- It does not claim that aged, coalesced, or thermally biased fog has the same tracer fidelity as freshly generated micron-scale fog.
- It does not guarantee a regulatory outcome; it provides the physics, peer-reviewed evidence, and validation controls needed for a technically defensible position.

3. Regulatory and Standards Context

3.1 FDA aseptic-processing guidance

FDA's aseptic-processing guidance states that HEPA-filtered air should maintain unidirectional airflow in critical areas and that in-situ air-pattern analysis should demonstrate unidirectional airflow and sweeping action under dynamic conditions. The guidance identifies approximately 0.45 m/s (90 ft/min) as a generally established velocity parameter in the critical area, while emphasizing that actual parameters must be justified and appropriate for each location. [1]

3.2 2026 FDA enforcement language

In the June 24, 2026 Wizcure Pharmaa warning letter and the July 2, 2026 International Medication Systems warning letter, FDA requested critical evaluation of airflow unidirectionality and thorough smoke studies under dynamic conditions. Both letters state that these studies should use proper practices and give "neutrally buoyant media" as an example to appropriately visualize airflow. [2,3]

The wording is significant in two ways. First, the regulatory objective stated in these letters is appropriate visualization of airflow, not selection of a named brand or generation method. Second, the parenthetical "e.g." introduces neutrally buoyant media as an example of proper practice rather than a technology-specific specification. In the cited letters, FDA does not state that ultrasonic atomization is prohibited, does not provide a particle-size cutoff, and does not mandate liquid nitrogen, glycol, mineral oil, or another specific tracer chemistry. This textual observation should not be interpreted as a guarantee that FDA will accept any particular device without supporting qualification data.

3.3 EU GMP Annex 1

EU GMP Annex 1 requires airflow patterns in cleanrooms and zones to be visualized, requires visualization studies where unidirectional airflow is required, and states that airflow-pattern studies should be performed both at rest and in operation, including simulated operator interventions, with video recordings retained. [4] This reinforces that the evidentiary endpoint is representative visualization of actual airflow behavior under operating conditions.

3.4 ISO 14644-3

ISO 14644-3:2019 provides cleanroom performance test methods for both unidirectional and non-unidirectional airflow and across as-built, at-rest, and operational states. Its public abstract also recognizes that multiple methods and apparatus can be appropriate for different end-use considerations and that alternative methods can be used by agreement. [5]

Source	Relevant expectation	Implication for tracer qualification
FDA 2004 aseptic guidance	Air-pattern analysis must demonstrate unidirectional flow/sweeping under dynamic conditions; ~0.45 m/s is a generally established critical-area velocity reference.	Performance of the airflow visualization is the endpoint.
FDA 2026 warning letters	Dynamic smoke studies; proper practices, with "neutrally buoyant media" given as an example.	No atomization method or droplet-size threshold specified.
EU GMP Annex 1 §4.15	Visualize airflow at rest and in operation; retain video; evaluate interventions.	Tracer must allow clear, representative observation.
ISO 14644-3:2019	Test methods for UDAF/non-UDAF and occupancy states; multiple/alternative methods recognized.	Method suitability should be justified for intended use.

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4. Scientific Evidence for Micron-Scale Water Fog as an Airflow Tracer

4.1 Direct ultrasonic-fog airflow visualization evidence

Kennedy (1987) directly evaluated ultrasonic water fog for airflow visualization in fume cupboards, microbiological safety cabinets, and extraction hoods. The study compared ultrasonic water fog with mineral-oil smoke and reported that the water fog demonstrated all airflow features visualized by the smoke. [6] This is unusually direct evidence because it compares the visible performance of ultrasonic water fog against an established smoke medium rather than relying only on theoretical calculations.

4.2 1.65 MHz ultrasonic atomization, 4–5 µm droplets, quantitative gas-flow measurement

Lozano et al. (2014) developed a high-flow-rate ultrasonic seeder specifically for gaseous-flow measurements. The unit used twelve piezoceramic disks oscillating at 1.65 MHz and generated water droplets with a Sauter mean diameter of approximately 4–5 µm. The authors described the requirement that seeding tracers be sufficiently small to follow the flow and demonstrated the ultrasonic seeder in a free air jet. The seeded flow was visualized and its three-dimensional velocity field was successfully measured by stereoscopic PIV. [7]

This paper is particularly relevant because both the actuation frequency and droplet size overlap the nominal operating regime of CRF-class ultrasonic fogging systems analyzed here. It establishes a peer-reviewed precedent for using 1.65 MHz ultrasonic atomization to generate 4–5 µm water droplets for quantitative air-velocity measurement—not merely qualitative visualization.

4.3 Six-micron water droplets validated as passive gas-phase tracers

Ayati et al. (2014) used approximately 6 µm water droplets as tracer particles in the gas phase of turbulent air/water pipe flow. The authors performed gas single-phase validation at Reynolds number 45,000 and compared measured results against direct numerical simulation, reporting very good agreement. In their concluding analysis they explicitly determined from Stokes-number analysis that the water droplets were small enough to be considered passive tracers. [8]

A 6 µm water droplet lies near the middle of the nominal 4–8 µm CRF range. This is strong experimental support that water droplets in this size regime can track gas-phase motion accurately enough for quantitative velocimetry.

4.4 Larger water fog droplets are routinely accepted as PIV air tracers when Stokes number is small

Tenhaus et al. (2024) seeded an airflow with approximately 12 µm fog droplets for high-resolution PIV above wind waves. The investigators calculated a particle relaxation time of 0.4 ms and an airflow time scale approximately thirty times larger, concluding that the fog particles were expected to closely follow the airflow streamlines because Stokes number was much less than one. [9]

Buckley et al. (2025) used approximately 15 µm fog microdroplets to obtain direct, millimeter-to-meter-scale observations of turbulent airflow above ocean waves. They explicitly assessed tracer suitability using Stokes number; the largest particles had a reported particle time scale of 1.2 ms versus an estimated Kolmogorov time scale of 23.6 ms, yielding St << 1. [10]

These studies do not prove every fogging system is suitable, but they provide an important scale comparison: rigorous PIV experiments have accepted water droplets larger than 8 µm as airflow tracers after showing adequate dynamic response. Smaller 4–8 µm droplets have still shorter response times and lower settling velocities, all else equal.

4.5 MHz ultrasonic atomization is a well-established mechanism for producing micrometer droplets

Modern ultrasonic-atomization research confirms that MHz-frequency actuation produces micrometer-diameter aerosols through high-frequency capillary-wave instability and ligament breakup. Shenoda et al. (2024) studied ultrasonic atomization at frequencies from 1 to 5 MHz and emphasized controlled production of micrometer-scale droplets, including operation above 1 MHz. [11] This supports the physical mechanism underlying MHz cleanroom foggers and is consistent with the 1.65 MHz, 4–5 µm gaseous-flow seeder demonstrated by Lozano et al. [7]

Study	Generation / medium	Droplet size	Application	Regulatory relevance
Kennedy, 1987 [6]	Ultrasonic nebulizer; water fog	Not central to abstract	Direct airflow visualization	Water fog reproduced airflow features shown by mineral-oil smoke.
Lozano et al., 2014 [7]	Ultrasonic; 1.65 MHz	4–5 µm SMD	Free air jet; stereoscopic PIV	Successful 3D velocity-field measurement.
Ayati et al., 2014 [8]	Water-droplet seeding	~6 µm mean	Turbulent air flow; PIV	Stokes analysis: droplets small enough to be passive tracers; validation vs DNS.
Tenhaus et al., 2024 [9]	Water fog	12 µm	Wind-wave tunnel air-side PIV	St << 1; particles expected to closely follow airflow streamlines.
Buckley et al., 2025 [10]	Fog microdroplets	~15 µm	In-situ ocean-surface airflow PIV	St << 1; suitability explicitly assessed.
Auvinen et al., 2022 [12]	Ultrasonic; 1.7 MHz	6–8 µm wet	Indoor dispersion study	Shows need to control evaporative-cooling plume bias; thermal correction used.

5. Airflow-Tracer Physics: Why Micron-Scale Droplets Follow Air

5.1 Particle relaxation time

When air velocity changes, a suspended droplet does not respond instantaneously because it has mass. In the low-Reynolds-number Stokes regime, particle relaxation time provides a first-order measure of how quickly a small spherical droplet adjusts toward the surrounding air velocity. Including the Cunningham slip correction for micron-scale particles:

Particle response equation

$$\tau_p = (\rho_p d^2 C_c) / (18 \mu)$$

where τ_p is particle relaxation time, ρ_p is droplet density, d is particle diameter, C_c is the Cunningham correction factor, and µ is air dynamic viscosity.

For water droplets between 4 and 8 µm at approximately room conditions, the calculated relaxation time is roughly 0.05–0.20 milliseconds. This is extremely short relative to the time scale of the cleanroom flow features ordinarily visualized by video. The particle therefore rapidly adapts to the local air velocity.

5.2 Stokes number

Stokes number compares the particle response time with the characteristic time scale of the flow:

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Stokes number

St = τp / τf

When St << 1, particle inertia is small relative to the flow time scale and tracer motion closely follows the carrier fluid. This flow-following requirement is foundational to PIV tracer selection and is explicitly applied in the cited experimental airflow studies. [8–10,13]

At U = 0.45 m/s, even using a small characteristic airflow length of only 10 mm gives calculated Stokes numbers of approximately 0.0023 for 4 µm, 0.0051 for 6 µm, and 0.0090 for 8 µm droplets. At larger 25 mm and 100 mm flow scales, the values are smaller still. These values are far below unity and are consistent with passive-tracer behavior.

5.3 Gravitational settling

Gravity creates downward slip even when particle inertia is otherwise negligible. In the Stokes regime, terminal settling velocity can be estimated from the particle relaxation time. The calculation below is intentionally conservative because it treats the droplet as a stable liquid sphere in still air; evaporation generally reduces droplet diameter and therefore reduces particle settling, while coalescence can increase diameter and settling.

Diameter (µm)	Relaxation time (ms)	Still-air settling (mm/s)	Settling / 0.45 m/s	Vertical drop while air travels 1 m (mm)
4	0.051	0.500	0.111%	1.11
5	0.079	0.775	0.172%	1.72
6	0.113	1.110	0.247%	2.47
7	0.154	1.505	0.335%	3.35
8	0.200	1.960	0.436%	4.36

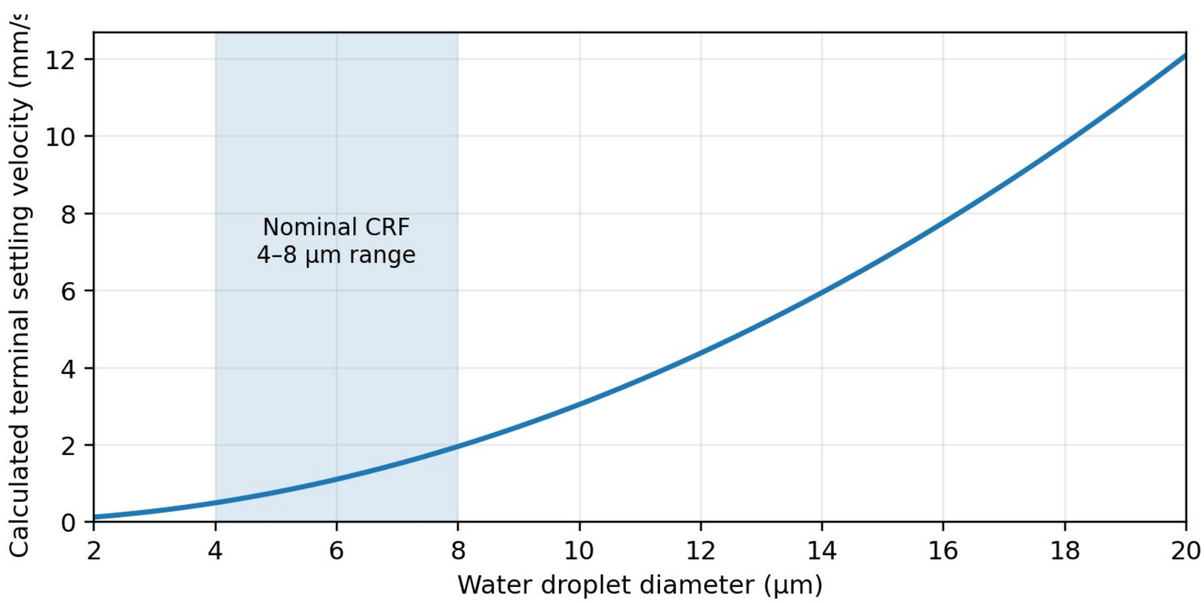

Figure 1. Calculated still-air terminal settling velocity versus water-droplet diameter using Stokes drag with Cunningham slip correction. Shaded band indicates the nominal 4–8 µm range analyzed in this paper. This is a physics calculation, not a measured CRF size-distribution curve.

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5.4 Scale interpretation for cleanroom visualization

At 0.45 m/s, air traverses 1 meter in about 2.22 seconds. An 8 µm water droplet with a calculated 1.96 mm/s terminal settling velocity would fall approximately 4.36 mm during that time in still-air-equivalent gravitational slip. A 4 µm droplet would fall approximately 1.11 mm. In a video intended to reveal macroscopic airflow direction, sweep, turbulence, ingress, or obstruction around aseptic equipment, this particle-scale slip is small relative to the meter-scale streamwise motion.

The relevant conclusion is not that settling equals zero. The conclusion is that, within the 4–8 µm range, particle-scale gravitational slip is small compared with typical unidirectional cleanroom airflow and particle response is fast compared with the time scale of the flow. This is precisely why similar-size water droplets have been used as quantitative air tracers in peer-reviewed PIV research. [7–10]

6. Critical Qualification Issue: Particle Behavior Is Not the Whole Plume

6.1 Evaporative cooling can create a false downward plume

A critical distinction must be made between the dynamics of an individual droplet and the buoyancy of the carrier-air/fog plume. Auvinen et al. (2022) used a commercial 1.7 MHz ultrasonic nebulizer that produced 6–8 µm wet particles. Immediately after release, evaporative cooling made the plume negatively buoyant and caused the surrounding air—and therefore the tracer cloud—to sink. The investigators mitigated the effect using a heated plate. [12]

This study is important because it demonstrates what a rigorous qualification must exclude. A 6–8 µm droplet can have excellent particle-scale response and still be carried in an artificial downward air mass if the generation system cools the carrier air. Conversely, a fogger that maintains release temperature near the surrounding air and introduces low momentum can preserve the natural flow field much more faithfully.

> **Why including contrary evidence makes the regulatory case stronger**
>
> The correct scientific position is not “ultrasonic fog is always neutrally buoyant.” That assertion is too broad and can be disproved by specific operating configurations.
>
> The defensible position is: “Micron-scale ultrasonic water fog is capable of passive-tracer behavior, and the complete fogging system can be qualified to verify that thermal and momentum biases are negligible for the intended smoke study.”

6.2 Source momentum can also distort the study

Any fog source can disturb airflow if the tracer is injected as a strong jet, if a wand is swept rapidly, or if the source is positioned so close to the critical feature that the source momentum dominates the local air motion. Recent FDA warning letters have criticized wand placement and movement as factors that precluded meaningful airflow assessment. [3] A qualified method should therefore demonstrate that the release technique does not create the pattern being observed.

6.3 Fog aging, coalescence, and evaporation

The nominal generated-droplet size describes fresh fog. During residence in air, droplets can evaporate, collide, coalesce, or be removed by surfaces. Evaporation decreases diameter and settling velocity but can cool the air if the latent-heat load is significant. Coalescence increases diameter and settling velocity. For this reason, airflow visualization should rely on freshly generated fog introduced near enough to the observation zone to provide visibility without intentionally accumulating an aged, highly concentrated cloud.

Sauter mean diameter (D32) should not be described as a “Sauter limit.” It is a statistical diameter representing the surface-area-to-volume relationship of a droplet distribution. It can be useful for characterizing an atomizer, but it is not a terminal size to which fog droplets grow.

7. Proposed Qualification Framework for Pharmaceutical Use

The following framework is designed to convert the peer-reviewed scientific basis into an auditable, site-specific demonstration that a particular ultrasonic fogger and study technique are fit for intended use. It should be incorporated into equipment qualification, the smoke-study SOP, or a supporting technical assessment, as appropriate to the site quality system.

Qualification element	Recommended evidence	Purpose
A. Droplet-size characterization	Document the generated droplet-size distribution using a suitable technique (e.g., laser diffraction, phase-Doppler measurement, or another justified aerosol-sizing method). Report distribution metrics such as D10/D50/D90 and/or D32, not only a single nominal value.	Confirms that the tested device operates in the micron-scale regime supported by tracer literature.
B. Release-temperature characterization	Measure fog/carrier-air temperature at or near the point of release and ambient air temperature under representative operating conditions, including warm-up and extended operation.	Detects thermal buoyancy that could cause artificial rising or sinking.
C. Still-air plume check	In a quiescent test space, record plume centroid behavior against a fixed reference grid. Evaluate whether any sustained rise/fall is attributable to thermal or source effects rather than expected particle settling.	Directly challenges the “not neutrally buoyant” concern for the specific device/configuration.
D. Source-momentum assessment	Document fan setting, hose/wand geometry and release technique. Where appropriate, compare local velocity with tracer on/off using a calibrated anemometer or independent velocimetry method.	Shows that fog introduction does not materially create or redirect the airflow pattern.
E. Dynamic airflow-study execution	Perform studies in the operational state with representative operator interventions, line activities, equipment configuration and obstruction conditions.	Directly aligns with current FDA and Annex 1 expectations.
F. Fresh-fog use window	Define operating technique that avoids excessive accumulation/aging and uses fresh visible tracer in the area being evaluated.	Reduces effects of coalescence and excessive evaporative cooling.
G. Water quality / residue control	Use DI, purified, sterile, or WFI water as required by site risk assessment; document cleaning/drying between uses.	Supports residue and contamination-control requirements.
H. Video quality and traceability	Use fixed camera positions when practical, appropriate contrast illumination, time/date identification, and retention of raw/unedited study video in accordance with site procedure.	Provides reviewable objective evidence and protects data integrity.

7.1 Recommended acceptance rationale

The acceptance criterion should be phrased in terms of intended-use fidelity rather than an impossible requirement of exact zero settling. A suitable technical rationale is that the tracer shall demonstrate negligible directional bias relative to the minimum airflow velocity and spatial resolution of the study, shall not introduce a material thermal plume or injection jet, and shall provide sufficient persistence and visibility to evaluate all critical airflow paths and dynamic interventions.

Where a site wants a quantitative margin, the facility should predefine and justify it based on its minimum qualified airflow velocity, camera resolution, critical-zone geometry, and the smallest airflow feature it must resolve. The supporting calculations in this paper provide a physics basis but should not substitute for device- and site-specific verification.

7.2 Recommended evidence package retained with each program

- Manufacturer technical specification for the fogger model and revision used.
- Executed droplet-size characterization or supplier certificate tied to the model/configuration.
- Fog outlet temperature / ambient comparison under representative runtime.
- Documented release configuration: outlet, hose, wand/nozzle, fan setting, fog output setting, and distance/orientation from the critical zone.
- Calibration status of airflow instruments used to establish the cleanroom operating condition.
- Approved smoke-study protocol defining interventions, camera views, acceptance criteria, and data-retention requirements.
- Raw video and final study report with deviations and quality approval.
- Change-control trigger requiring reassessment when fogger hardware, transducers, controls, hoses/nozzles, operating settings, or study technique materially change.

8. Application to CRF-Class Ultrasonic Cleanroom Foggers

8.1 Nominal operating basis used for this analysis

Applied Physics CRF-class ultrasonic cleanroom foggers are designed to generate visible water fog for cleanroom airflow visualization. For the aerodynamic analysis in this paper, the nominal generated-droplet range is 4–8 µm and the ultrasonic transducer operating frequency is approximately 1.65 MHz. These values are treated as manufacturer nominal design values for the analyzed configuration. A regulatory technical file should distinguish nominal manufacturer specifications from independently measured lot/model data.

Evidence hierarchy for CRF use

Level 1 - Independent peer-reviewed science: establishes that ultrasonic atomization in the 1.65 MHz range can produce 4–5 µm water droplets suitable for gas-flow PIV, and that ~6 µm water droplets can behave as passive gas tracers. [7,8]

Level 2 - First-principles aerodynamic analysis: establishes expected response time, Stokes number, and gravitational settling for a stated droplet-size range.

Level 3 - Model/configuration qualification: confirms that the actual CRF unit, hose/wand, operating setting, water, and room conditions do not introduce unacceptable thermal or momentum bias. This is the decisive device-specific layer for an FDA-facing technical file.

8.2 Alignment with published scientific evidence

The nominal CRF frequency and size regime align closely with Lozano et al.'s peer-reviewed 1.65 MHz ultrasonic seeder producing 4–5 µm water droplets for gaseous-flow PIV. [7] The CRF nominal size range also encompasses the approximately 6 µm water droplets used and validated as passive gas-phase PIV tracers by Ayati et al. [8] Meanwhile, peer-reviewed PIV studies have accepted larger 12 µm and approximately 15 µm water-fog droplets after confirming Stokes-number suitability. [9,10]

This evidence is stronger than a general manufacturer statement that the fog "floats." It demonstrates that the same physical droplet-size regime has been used by independent researchers to measure actual air velocity fields.

8.3 Recommended wording for CRF technical documentation

Recommended manufacturer statement

The CRF ultrasonic fogger generates a micron-scale water aerosol intended for airflow visualization. In the nominal 4–8 µm generated-droplet range, calculated particle inertia and gravitational settling are small relative to typical cleanroom unidirectional airflow, and peer-reviewed gas-flow research has demonstrated passive-tracer behavior for water droplets in the same size regime. The system should be used and qualified in a manner that minimizes thermal and injection-momentum bias and uses fresh fog for the observation interval.

8.4 Data that would further strengthen the manufacturer technical file

The scientific literature already provides a strong basis for suitability; however, the strongest possible manufacturer dossier would add direct CRF-specific metrology. Applied Physics should maintain or commission the following independent or traceable measurements for each current production platform:

1. Droplet-size distribution measured at the fog outlet and, optionally, at defined downstream distances, with documented water quality, temperature, humidity, fan setting, and fog-output setting.
2. Fog/carrier-air outlet temperature versus ambient throughout a representative operating cycle.
3. Source-velocity profile at representative outlet and wand configurations, including the lowest practical setting for critical-zone visualization.
4. Still-air video characterization of plume centroid rise/fall using a fixed grid.
5. Side-by-side airflow visualization against an independent accepted tracer or PIV reference in a controlled flow field, demonstrating concordant direction, recirculation, and obstruction behavior.

These tests are recommended because they turn a general scientific argument into direct evidence about the exact commercial device being presented to an investigator. In a regulatory dispute, model-specific data are harder to dismiss than a brochure claim or a literature-only analogy.

9. Technical Response to the Proposition That "Ultrasonic Fog Is Not Neutrally Buoyant"

A categorical rejection of ultrasonic fog based solely on the atomization mechanism is not supported by the cited aerosol and flow-measurement literature. The following points constitute the core scientific response:

1. Ultrasonic water fog has been directly compared with conventional smoke for airflow visualization and was reported to demonstrate the same airflow features. [6]
2. A peer-reviewed 1.65 MHz ultrasonic device has generated 4–5 µm water droplets specifically for gaseous-flow seeding and successfully supported three-dimensional PIV velocity measurements. [7]
3. Approximately 6 µm water droplets have been validated experimentally as passive gas-phase tracers, including comparison of PIV measurements to DNS and Stokes-number analysis. [8]
4. Water-fog droplets of 12–15 µm—larger than the nominal CRF range—have been used in modern high-resolution airflow PIV after explicit Stokes-number assessment showed appropriate flow following. [9,10]

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5. For 4–8 µm water droplets, calculated terminal settling velocity is approximately 0.50–1.96 mm/s and particle relaxation time approximately 0.05–0.20 ms at room conditions. At 0.45 m/s airflow, that settling velocity is approximately 0.11–0.44% of the streamwise velocity.

6. The genuine technical risks are plume-scale thermal buoyancy and source momentum, not ultrasonic atomization per se. Peer-reviewed evidence shows these effects can occur and therefore should be directly qualified. [12]

7. FDA's recent warning-letter language requires appropriate airflow visualization and gives neutrally buoyant media as an example of proper practice; the letters do not prescribe a generation technology or a literal density-equality test. [2,3]

Scientifically precise conclusion

A properly qualified 4–8 µm ultrasonic water-fog system can function as an adequate passive airflow tracer for pharmaceutical smoke studies. The phrase "neutrally buoyant" should be understood operationally as negligible tracer slip and negligible generation-induced bias over the study scale—not as literal equality of liquid-water and air density.

10. Recommended Smoke-Study Technique Using Ultrasonic Water Fog

Tracer selection is only one component of a defensible airflow-visualization program. Current FDA observations demonstrate that poor execution can invalidate a study even when the tracer itself is appropriate. The following practices should be incorporated into the SOP and protocol:

1. Establish and document that the HVAC/critical-zone airflow is within its qualified operating range before visualization begins.

2. Configure the cleanroom, RABS, isolator, line, guards, doors, transfer systems, equipment, and personnel as they are during the actual operation being qualified.

3. Introduce fog slowly and locally enough to reveal the existing airflow rather than create a jet. Avoid rapid sweeping motions that obscure the path or inject excessive momentum.

4. Start upstream of the critical region and visualize first air, sweep over and away from exposed sterile product/components, potential reflux, turbulence around obstructions, and the effect of all representative interventions.

5. Use camera angles and contrast lighting that keep the critical airflow path visible. Avoid blocking the view with the operator, wand, or fogger.

6. Where the tracer is introduced through a long hose or wand, qualify the actual configuration because hose length, diameter, fan setting, and bends can alter release velocity and residence time.

7. Use fresh fog; avoid intentionally flooding the room until droplets have aged or coalesced. If high fog density is required for visibility, establish an operating technique that remains within the qualified thermal and concentration envelope.

8. Record deviations and repeat views when the tracer source itself appears to perturb the air or when visibility is insufficient to make a reliable conclusion.

9. Retain raw video and document the evaluator's interpretation of each critical view, including expected flow, observed flow, intervention, acceptance criterion, and disposition.

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11. Conclusions

The scientific literature supports the use of micron-scale water droplets as gas-phase airflow tracers. The evidence includes direct comparison of ultrasonic water fog with conventional smoke for airflow visualization, 1.65 MHz ultrasonic generation of 4–5 µm water droplets for successful stereoscopic PIV, approximately 6 µm water droplets validated as passive gas-phase tracers, and modern PIV studies using even larger 12–15 µm fog droplets after demonstrating Stokes-number suitability. [6–10]

For a nominal 4–8 µm water-droplet range, first-principles calculations show very short aerodynamic response times and low gravitational settling relative to typical cleanroom unidirectional airflow. These properties are consistent with faithful streamline following over normal visualization distances and times.

The strongest technical position is therefore not an absolute claim that every ultrasonic fog plume is inherently neutrally buoyant. Peer-reviewed evidence shows that evaporative cooling can cause a negatively buoyant carrier-air plume in certain configurations. [12] Instead, the scientifically robust position is that ultrasonic atomization is capable of producing an appropriate passive tracer, and suitability is demonstrated by qualification of droplet size, thermal behavior, injection momentum, fog age/concentration, and representative dynamic study technique.

On that evidence, a properly qualified CRF-class ultrasonic fogging system operating in the 4–8 µm generated-droplet regime is scientifically suitable for pharmaceutical airflow visualization studies. A blanket exclusion based solely on ultrasonic generation is not supported by the current peer-reviewed evidence reviewed in this paper.

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Appendix A. Calculation Basis and Worked Examples

A.1 Assumptions

- Water droplet density ρ_p = 998.2 kg/m³ (representative near 20°C).
- Air density ρ_a = 1.204 kg/m³ and dynamic viscosity $\mu = 1.81 \times 10^{-5}$ Pa·s (representative near 20°C, atmospheric pressure).
- Gravitational acceleration g = 9.80665 m/s².
- Mean free path of air approximately 0.066 µm for Cunningham slip-correction estimate.
- Spherical droplets and low particle Reynolds number; no electric field or unusual electrostatic charging.
- Reference unidirectional airflow U = 0.45 m/s for scale comparison, consistent with the generally established FDA guidance reference; site-specific velocities control actual qualification. [1]

A.2 Equations

Cunningham correction: $C_c = 1 + (2\lambda/d)[1.257 + 0.4 \exp(-1.1d/(2\lambda))]$

Particle relaxation time: $\tau_p = \rho_p d^2 C_c / (18\mu)$

Terminal settling velocity: $v_s \approx \tau_p g(1 - \rho_a/\rho_p)$

Characteristic flow time: $\tau_f = L/U$

Stokes number: $St = \tau_p/\tau_f$

A.3 Stokes-number examples

Droplet (µm)	St at L=10 mm	St at L=25 mm	St at L=100 mm
4	0.00230	0.00092	0.00023
6	0.00510	0.00204	0.00051
8	0.00901	0.00360	0.00090

All example Stokes numbers are far below unity. Because Stokes number depends on the actual flow time scale, a site or investigator performing a quantitative analysis should select L and U that represent the smallest relevant airflow feature and velocity for the study.

A.4 Interpretation limitations

These calculations characterize particle-scale inertia and settling only. They do not predict carrier-plume buoyancy caused by temperature, humidity, evaporation, or fog mass loading, and they do not quantify source-jet momentum. Those effects must be addressed experimentally as described in Section 7.

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Appendix B. Evidence Strength and Applicability Assessment

Proposition	Primary evidence	Strength	Applicability / limitation
Direct equivalence of ultrasonic water fog to visual smoke features	Kennedy 1987 [6]	High	Direct qualitative airflow-visualization comparison; older study, not pharmaceutical aseptic filling.
Ultrasonic 1.65 MHz generation of 4–5 µm water droplets for gas-flow measurement	Lozano 2014 [7]	Very high	Near-exact frequency/size analog; quantitative PIV application.
~6 µm water droplets as passive gas-phase tracers	Ayati 2014 [8]	Very high	Experimental validation against DNS plus Stokes-number conclusion.
12–15 µm water fog as PIV airflow tracers	Tenhaus 2024; Buckley 2025 [9,10]	High	Modern high-resolution air-flow measurement; larger droplets than CRF nominal range.
MHz ultrasound produces micrometer aerosols	Shenoda 2024 [11]	High	Modern mechanistic atomization research; not cleanroom-specific.
Flow-tracer fidelity is governed by particle response to carrier-fluid motion	Melling 1997 [13]	High / foundational	Widely cited PIV tracer-selection framework; supports response-time/Stokes analysis.
Thermal bias can cause ultrasonic plume to sink	Auvinen 2022 [12]	Very high for limitation	Direct counterexample; supports need for thermal qualification rather than categorical acceptance.
Current regulatory expectation for neutral/representative smoke media	FDA 2026 [2,3]	Very high	Direct current enforcement language; does not define an atomization technology.
Dynamic visualization and retained video	EU Annex 1 [4]	Very high	Direct current GMP expectation.

Appendix C. Suggested Technical Language for a Regulatory Response

The following language is provided as a technical template only and should be adapted to the facility's specific observation, CAPA, executed data, and quality/legal review:

Suggested response text

The airflow-visualization medium used in our study is a micron-scale water aerosol generated by ultrasonic atomization. We evaluated the scientific suitability of this tracer rather than relying on the atomization method alone. Peer-reviewed literature demonstrates that ultrasonic water fog can reproduce airflow features visualized by conventional smoke; a 1.65 MHz ultrasonic seeder has generated 4–5 µm water droplets for successful stereoscopic PIV measurement of gaseous flow; and approximately 6 µm water droplets have been experimentally validated as passive gas-phase tracers by Stokes-number analysis and comparison to DNS.

For the 4–8 µm generated-droplet range used in our technical assessment, calculated particle response time is approximately 0.05–0.20 ms and still-air settling velocity approximately 0.50–1.96 mm/s. At a 0.45 m/s airflow reference velocity, this represents approximately 0.11–0.44% gravitational slip relative to the streamwise velocity. We recognize that particle size alone is not sufficient: evaporative cooling or injection momentum can bias a tracer plume. Accordingly, our qualification controls include [insert executed site-specific droplet-size, outlet-temperature, source-momentum/still-air behavior, and study-method evidence].

On the basis of the cited literature and our executed qualification, we consider the tracer functionally neutrally buoyant for the intended airflow-visualization interval—that is, its particle inertia and gravitational slip are negligible relative to the airflow being evaluated and the generation system does not introduce a material thermal or momentum bias. Our revised/confirmed study is performed under representative dynamic conditions and is designed to demonstrate first-air protection and airflow unidirectionality throughout critical processing and interventions.

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12. References

[1] U.S. Food and Drug Administration. Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice: Guidance for Industry. October 2004. Link

[2] U.S. Food and Drug Administration. Warning Letter: Wizcure Pharmaa Private Limited, MARCS-CMS 726378. June 24, 2026. Link

[3] U.S. Food and Drug Administration. Warning Letter: International Medication Systems Limited, MARCS-CMS 727560. July 2, 2026. Link

[4] European Commission. EudraLex Volume 4, EU Guidelines for Good Manufacturing Practice, Annex 1: Manufacture of Sterile Medicinal Products. 2022 revision; in operation 25 August 2023 (8.123 from 25 August 2024). Link

[5] International Organization for Standardization. ISO 14644-3:2019, Cleanrooms and associated controlled environments — Part 3: Test methods. Link

[6] Kennedy DA. Water fog as a medium for visualization of airflows. The Annals of Occupational Hygiene. 1987;31(2):255–259. doi:10.1093/annhyg/31.2.255. Link

[7] Lozano A, González-Espinosa A, García JA, Calvo E, Barroso J, Barreras F. High flow-rate ultrasonic seeder. Flow Measurement and Instrumentation. 2014;38:62–66. doi:10.1016/j.flowmeasinst.2014.04.002. Link

[8] Ayati AA, Kolaas J, Jensen A, Johnson GW. A PIV investigation of stratified gas–liquid flow in a horizontal pipe. International Journal of Multiphase Flow. 2014;61:129–143. doi:10.1016/j.ijmultiphaseflow.2014.01.008. Link

[9] Tenhaus J, Buckley MP, Matt S, Savelyev IB. Viscous and turbulent stress measurements above and below laboratory wind waves. Experiments in Fluids. 2024;65:174. doi:10.1007/s00348-024-03898-7. Link

[10] Buckley MP, Horstmann J, Savelyev I, Carpenter JR. Direct observations of airflow separation over ocean surface waves. Nature Communications. 2025;16:5526. doi:10.1038/s41467-025-61133-1. Link

[11] Shenoda A, Brenker J, Alan T. High-precision ultrasonic atomization using oscillating microchannels: Interplay of three-dimensional vibrational modes and droplet ejection mechanisms. Physics of Fluids. 2024;36(9):092025. doi:10.1063/5.0225545. Link

[12] Auvinen M, Kuula J, Grönholm T, Sühring M, Hellsten A. High-resolution large-eddy simulation of indoor turbulence and its effect on airborne transmission of respiratory pathogens—Model validation and infection probability analysis. Physics of Fluids. 2022;34(1):015124. doi:10.1063/5.0076495. Link

[13] Melling A. Tracer particles and seeding for particle image velocimetry. Measurement Science and Technology. 1997;8(12):1406–1416. doi:10.1088/0957-0233/8/12/005. Link

Source-selection note: Regulatory references are primary agency/standards sources. Scientific references are peer-reviewed journal articles or publisher-hosted records. The paper intentionally includes evidence that identifies a limitation of ultrasonic aerosol release (evaporative-cooling plume bias) because a defensible regulatory assessment must address contrary evidence rather than omit it.

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