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TECHNICAL SUBSTANTIATION REPORT

CRF6 Technical Substantiation Report: Functional Neutral Buoyancy and Airflow-Tracer Fidelity of Micron-Scale Ultrasonic Water Fog

Scientific evidence, first-principles analysis, FDA regulatory context, CRF6 design basis, limitations, and the qualification path required for pharmaceutical smoke studies

Prepared by Applied Physics

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External use: client-facing technical support document intended for quality, validation, engineering, regulatory, and FDA inspection-response packages.

Document purpose	Client-facing scientific and engineering support for pharmaceutical airflow-visualization qualification and regulatory correspondence.
Regulatory posture	Supports a scientifically defensible suitability rationale; does not claim FDA approval, certification, or site-specific compliance.
Controlled claim	Ultrasonic atomization can produce micron-scale water droplets that function as passive/operationally neutrally buoyant airflow tracers when the complete release system is qualified.
Device-specific status	CRF6 nominal design basis is approximately 1.65 MHz and 4-8 micrometer fresh generated droplets. Direct finished-device metrology is addressed by the companion qualification protocol.

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1. Executive Regulatory Position

The central technical question is not whether a liquid-water droplet has the same material density as air. It does not. The relevant question for an airflow-visualization study is whether the tracer faithfully follows the local air motion over the spatial and temporal scale being evaluated, without introducing a material gravitational, inertial, thermal, or source-momentum bias.

Peer-reviewed evidence directly supports ultrasonic water fog as an airflow-visualization medium. Kennedy reported that water fog produced by an ultrasonic nebulizer was suitable for airflow visualization and demonstrated the airflow features visualized by mineral-oil smoke. Lozano et al. developed a 1.65 MHz ultrasonic seeder that generated 4-5 micrometer Sauter-mean-diameter water droplets and successfully measured a three-dimensional gaseous velocity field using stereoscopic particle image velocimetry (PIV). Ayati et al. used approximately 6 micrometer water droplets as passive tracers in the gas phase and validated the method against independent flow data. [6-8]

FDA's 2026 warning-letter language has intensified scrutiny of smoke-study execution and specifically gives "neutrally buoyant media" as an example of proper practice. The cited letters require appropriate visualization of airflow under dynamic conditions but do not prescribe a specific fog-generation technology or state that ultrasonic atomization is prohibited. [2-4]

> **Regulatory conclusion: the scientific literature does not support a categorical proposition that an airflow tracer is unsuitable merely because it was generated ultrasonically. Suitability must be determined from tracer size, response time, gravitational slip, plume thermal behavior, source momentum, visibility, and the executed study configuration.**

2. Scope and Intended Use

This report addresses Applied Physics CRF6 ultrasonic cleanroom fogging systems used to visualize airflow in pharmaceutical and biotechnology environments, including ISO 5/Grade A critical zones, RABS, isolators, laminar-flow workstations, cleanrooms, and associated airflow studies. It is intended to support technical assessments, qualification packages, CAPA responses, and regulatory correspondence when accompanied by site-specific executed evidence.

- The report supports scientific suitability of micron-scale ultrasonic water fog as a passive airflow tracer when the complete system is qualified.
- It does not state that liquid water has the same density as air.
- It does not state that every ultrasonic fogger or every hose/wand/fan configuration is automatically suitable.
- It does not substitute for a facility-specific dynamic airflow-visualization study.

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- It does not represent FDA approval, certification, or endorsement.

3. FDA Regulatory Context

3.1 FDA aseptic-processing guidance

FDA's aseptic-processing guidance states that HEPA-filtered air in critical areas should be supplied at a velocity sufficient to sweep particles away from exposed product and maintain unidirectional airflow during operations. It further states that in-situ air-pattern analysis should demonstrate unidirectional airflow and sweeping action over and away from product under dynamic conditions, including the impact of interventions and equipment design. FDA notes that video recording is useful for evaluating airflow and configuration changes. [1]

The same guidance notes that 0.45 m/s (90 ft/min), plus or minus 20 percent around the setpoint, has generally been established as a reference velocity, while emphasizing that the velocity established for each processing line should be justified. [1]

3.2 2026 enforcement emphasis

In the June 24, 2026 Wizcure Pharmaa warning letter and the July 2, 2026 International Medication Systems warning letter, FDA instructed the firms to conduct thorough dynamic smoke studies using proper practices, giving "neutrally buoyant media" as the example for appropriately visualizing airflow. [2,3]

FDA has also criticized execution issues independent of tracer type. In May 2026, FDA cited rapid manual movement of the smoke source and obstruction of visibility in the Sato Pharmaceutical warning letter. This reinforces that tracer injection technique and camera visibility are integral to study validity. [4]

> **The regulatory objective is accurate visualization of actual airflow. The atomization mechanism is one input to tracer behavior, not the acceptance criterion by itself.**

4. Scientific Evidence Supporting Ultrasonic Water Fog

4.1 Direct comparison of ultrasonic water fog with conventional smoke

Kennedy (1987) directly studied water fog produced by an ultrasonic nebulizer for airflow visualization in fume cupboards, microbiological safety cabinets, and extraction hoods. The study reported that water fog was suitable for airflow visualization and demonstrated the airflow features visualized by mineral-oil smoke. [6]

4.2 1.65 MHz ultrasonic atomization used for quantitative gas-flow velocimetry

Lozano et al. (2014) designed an ultrasonic seeder using twelve piezoceramic disks at approximately 1.65 MHz. With water, the device generated droplets with Sauter mean diameter of

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approximately 4-5 micrometers. The seeded gas flow was visualized and the three-dimensional velocity field was successfully measured using stereoscopic PIV. The paper explicitly frames adequate tracer size as a requirement for following gaseous flow. [7]

4.3 Six-micrometer water droplets validated as gas-phase tracers

Ayati et al. (2014) performed PIV measurements of turbulent stratified air/water flow using approximately 6 micrometer water droplets as gas-phase tracer particles. The study used the water droplets specifically as passive tracers and validated the gas-phase measurements against independent flow data. [8]

4.4 Tracer-particle physics is governed by response, not material density alone

PIV literature evaluates tracer suitability by whether the particle response time is short compared with the characteristic time scale of the flow. Melling's review describes the need to choose tracer particles small enough to follow gas motion accurately. In engineering practice, Stokes number is the standard non-dimensional measure of that response. A Stokes number far below unity indicates low particle inertia relative to the flow feature being observed. [9]

5. Ultrasonic Frequency and Droplet Size

Ultrasonic atomization at MHz frequencies is a well-established mechanism for producing micrometer-scale droplets. The relationship is not a single universal calibration because transducer geometry, drive amplitude, liquid depth, liquid properties, airflow, and atomizer configuration also matter. Nevertheless, independent measurements demonstrate the expected inverse relationship between drive frequency and generated droplet diameter.

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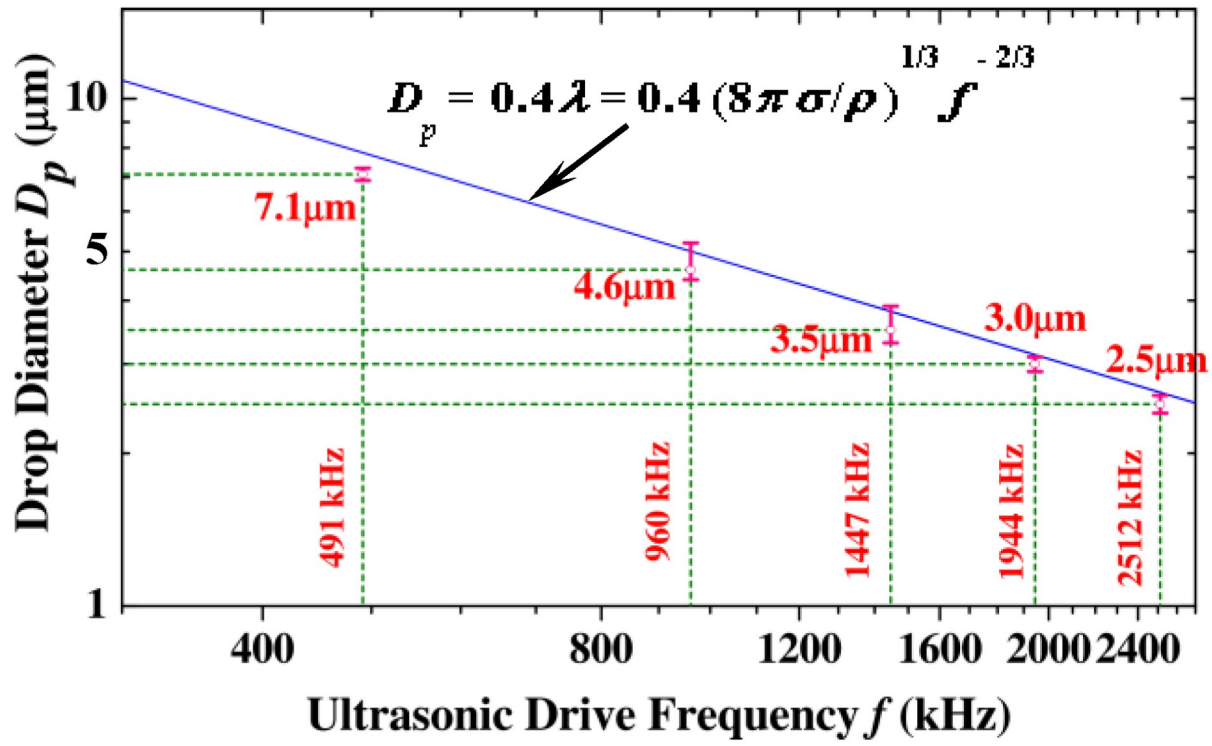


Figure 1. Experimental droplet diameter versus ultrasonic drive frequency. Reproduced from Tsai et al., Micromachines 2017, 8, 56, Figure 9, under CC BY 4.0. This is independent scientific evidence and is not a CRF6 calibration curve.

The figure is valuable because it independently demonstrates the physics of MHz ultrasonic atomization, but it should not be used to infer an exact CRF6 size distribution from frequency alone. Finished-device droplet-size characterization remains the stronger evidence.

6. CRF6 Design Basis

For the CRF6 configuration addressed by this report, Applied Physics uses a nominal ultrasonic drive frequency of approximately 1.65 MHz and a nominal fresh generated-droplet size range of approximately 4-8 micrometers. These values are manufacturer design-basis values pending the finished-device verification steps defined in AP-QP-CRF6-001.

The public technical position is intentionally based on finished-device performance rather than the identity or country of origin of an internal component supplier. Component traceability remains part of the controlled manufacturing record and can be addressed separately if specifically required by a customer quality agreement or regulatory request.

The 1.65 MHz / 4-8 micrometer CRF6 design basis overlaps the peer-reviewed 1.65 MHz / 4-5 micrometer ultrasonic PIV seeder reported by Lozano et al. and the approximately 6 micrometer water-droplet gas tracer used by Ayati et al. [7,8]

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7. First-Principles Tracer-Fidelity Analysis

7.1 Particle relaxation time

For small spherical water droplets in air, the aerodynamic response time scales approximately with the square of droplet diameter. Using representative room-condition properties, the calculated relaxation time is approximately 0.051 ms for a 4 micrometer droplet and 0.200 ms for an 8 micrometer droplet. These response times are extremely short relative to the time scale of cleanroom airflow structures normally resolved by smoke-study video.

7.2 Gravitational settling

Droplet	Response time	Still-air settling	Settling / 0.45 m/s	Drop while air travels 1 m	Interpretation
4 µm	0.051 ms	0.50 mm/s	0.11%	1.11 mm	Very low gravitational slip
6 µm	0.113 ms	1.11 mm/s	0.25%	2.47 mm	Very low gravitational slip
8 µm	0.200 ms	1.96 mm/s	0.44%	4.36 mm	Low gravitational slip

These calculations do not claim zero settling. They show that particle-scale settling is small relative to a representative 0.45 m/s unidirectional airflow. The practical criterion is whether the tracer path is materially biased relative to the airflow feature being evaluated.

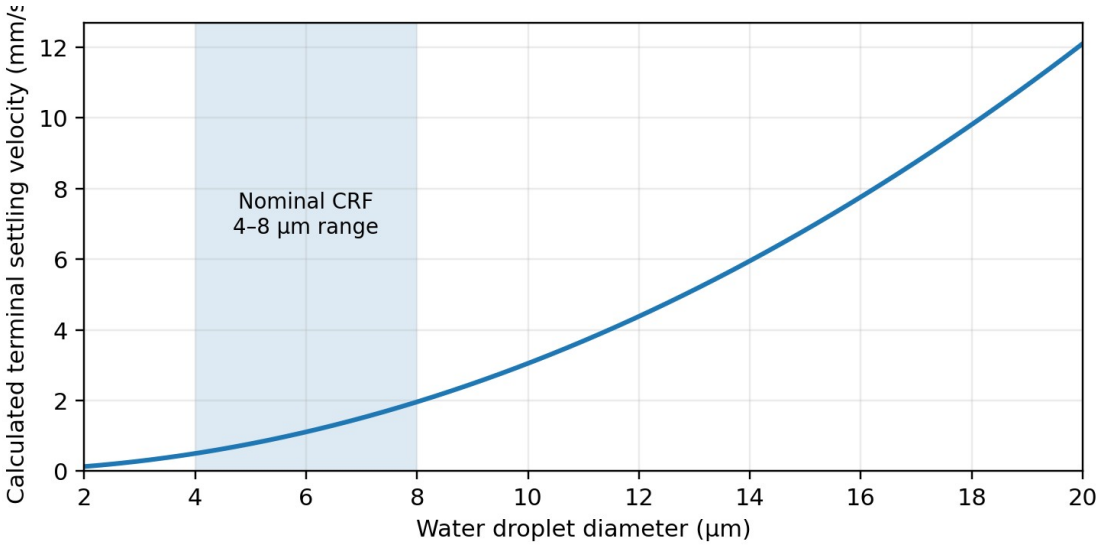


Figure 2. Calculated still-air terminal settling velocity versus water-droplet diameter. The 4-8 micrometer band represents the CRF6 nominal design basis. This is a physics calculation, not a finished-device particle-size measurement.

7.3 Stokes-number interpretation

At U = 0.45 m/s and using a deliberately small characteristic airflow length of 10 mm, the calculated Stokes numbers are approximately 0.0023, 0.0051, and 0.0090 for 4, 6, and 8 micrometer droplets, respectively. These values are far below unity and are consistent with rapid following of the local gas motion. Larger cleanroom flow length scales reduce Stokes number further.

8. The Critical Limitation: Plume-Scale Buoyancy

Particle-scale tracer fidelity is necessary but not sufficient. The complete fog plume can be thermally biased even when the individual droplets are small. Auvinen et al. used a 1.7 MHz ultrasonic nebulizer that produced 6-8 micrometer wet particles and observed immediate negative buoyancy caused by evaporative cooling of the carrier air. The authors reduced the artifact by thermally conditioning the release region. [10]

This study is not evidence that ultrasonic atomization is inherently unsuitable. It is evidence that a robust qualification must measure the actual plume temperature and still-air vertical drift of the finished fog-delivery configuration. The same principle applies to any tracer source that can add heat, cold, or injection momentum.

> **A defensible FDA position should not deny the existence of thermal-plume effects. It should show that the CRF6 configuration used for the study has been tested and does not introduce a material thermal or momentum bias.**

9. Why "Functional Neutral Buoyancy" Is the Correct Engineering Term

Strict hydrostatic neutral buoyancy means density equality between an object and the surrounding fluid. A liquid-water droplet cannot literally have the density of room air. In airflow visualization, however, the functional requirement is tracer fidelity: the visible media should move with the airflow closely enough that observed direction, sweeping action, eddies, reflux, turbulence, obstruction effects, and intervention effects represent the air rather than the tracer source.

For this report, functional neutral buoyancy means: gravitational, inertial, thermal, and source-momentum deviations are sufficiently small, over the intended observation interval and spatial scale, that the tracer faithfully represents the local airflow. This definition is testable and is aligned with the actual purpose of a pharmaceutical smoke study.

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10. CRF6 Technical Substantiation Matrix

Claim / parameter	Independent evidence	CRF6 basis	Current status	Regulatory implication
Ultrasonic fog can visualize airflow	Kennedy directly compared ultrasonic water fog with mineral-oil smoke. [6]	Ultrasonic water fog	Scientifically supported	Atomization mechanism alone is not a scientific basis for rejection.
1.65 MHz can generate micron-scale tracer droplets	Lozano: 1.65 MHz, 4-5 µm SMD, successful stereoscopic PIV. [7]	~1.65 MHz nominal	Scientific + manufacturer design basis	Direct CRF6 frequency verification closes the device-specific gap.
4-8 µm droplets can follow gas flow	Ayati: ~6 µm water droplets as passive gas tracer. [8]	4-8 µm nominal	Scientific + manufacturer design basis	Direct CRF6 D10/D50/D90/D32 measurement closes the device-specific gap.
Particle gravitational slip is small	Stokes settling calculation	0.50-1.96 mm/s for 4-8 µm	Calculated	Supports functional neutral-buoyancy rationale at typical cleanroom velocity.
Plume thermal bias must be controlled	Auvinen: evaporative cooling caused negative buoyancy. [10]	Not yet independently measured in this report	Requires qualification	Measure outlet/plume temperature and still-air drift.
Source must not disturb airflow	FDA warning letters criticize source technique and visibility. [2-4]	Depends on hose/wand/fan setting	Requires configuration qualification	Qualify actual release geometry and document slow, non-disruptive technique.

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Claim / parameter	Independent evidence	CRF6 basis	Current status	Regulatory implication
Dynamic study execution	FDA requires dynamic evaluation of interventions. [1-4]	Site responsibility	Site-specific	White paper supports tracer; site protocol/report establishes compliant execution.

11. Device-Specific Evidence Required to Complete the Strongest FDA Package

The literature and calculations establish the scientific mechanism. To make the evidentiary package difficult to challenge on device-specific grounds, Applied Physics should execute the companion qualification protocol AP-QP-CRF6-001 and retain the resulting raw data. The highest-value tests are:

1. Electrical frequency verification of the actual CRF6 driver/transducer system under representative operating load.
2. Finished-device droplet-size distribution at the outlet and representative downstream locations using a validated method, reporting D10, D50, D90, and D32 where technically applicable.
3. Fog/plume temperature versus ambient throughout representative operation and across expected water temperature/humidity conditions.
4. Still-air plume vertical-drift test after initial source momentum has decayed.
5. Outlet/wand source-velocity characterization for each qualified hose/nozzle/fan configuration.
6. Comparative airflow-tracer study against an accepted reference tracer in a controlled flow field, with predefined concordance criteria.
7. Dynamic study execution controls: interventions, camera angles, visibility, source movement, configuration traceability, raw video retention, deviations, and Quality approval.

12. Recommended Client/FDA Position Statement

Based on peer-reviewed airflow-visualization and PIV literature, first-principles aerosol dynamics, and the CRF6 nominal operating regime, Applied Physics concludes that micron-scale ultrasonic water fog is scientifically capable of functioning as a passive, functionally neutrally buoyant airflow tracer. Ultrasonic atomization is not, by itself, a scientifically valid basis for determining tracer unsuitability. Suitability of the specific CRF6 configuration should be demonstrated

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through documented finished-device and site-specific qualification addressing droplet size, plume thermal behavior, source momentum, visibility, and dynamic study execution.

A client should avoid stating that this report is an FDA approval or that use of a CRF6 automatically makes a smoke study compliant. The defensible statement is that the tracer technology has a scientific basis for suitability and that the facility has qualified the actual configuration and study execution for its intended use.

13. Conclusions

The evidence reviewed in this report supports three distinct conclusions. First, ultrasonic water fog has been directly demonstrated as a medium for airflow visualization. Second, a 1.65 MHz ultrasonic seeder producing 4-5 micrometer water droplets has been used successfully for quantitative gas-flow PIV, and approximately 6 micrometer water droplets have independently been used as passive gas-phase tracers. Third, first-principles calculations for 4-8 micrometer water droplets show very short response times and low gravitational settling relative to typical cleanroom unidirectional airflow. [6-9]

The strongest position is therefore not an absolute claim that every ultrasonic plume is automatically neutrally buoyant. The scientifically robust position is that ultrasonic atomization can produce a functionally neutrally buoyant/passive airflow tracer, while finished-device qualification must rule out thermal and momentum artifacts. A categorical exclusion based solely on ultrasonic generation is not supported by the scientific evidence reviewed here.

Appendix A. Calculation Basis

- Water density: 998.2 kg/m³ (representative near 20 °C).
- Air density: 1.204 kg/m³; dynamic viscosity: 1.81×10^{-5} Pa·s (representative near 20 °C at atmospheric pressure).
- Gravitational acceleration: 9.80665 m/s².
- Small spherical droplets in the low-particle-Reynolds-number regime.
- Reference airflow velocity for comparison: 0.45 m/s. Actual site-qualified velocity controls the site-specific assessment.

Particle relaxation time: $\tau p \approx \rho p\, d^2\, Cc / (18\, \mu)$.

Terminal settling velocity: $vs \approx \tau p\, g\, (1 - \rho a/\rho p)$.

Stokes number: $St = \tau p / \tau f$, where $\tau f = L/U$.

These equations characterize particle-scale behavior and do not predict plume thermal buoyancy

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or source-jet momentum.

Appendix B. Evidence Strength Assessment

Evidence	Strength	Direct relevance	Key limitation
Kennedy ultrasonic water-fog airflow visualization	High	Direct ultrasonic fog vs smoke visualization	Older study; not pharmaceutical aseptic line specific
Lozano 1.65 MHz / 4-5 µm ultrasonic PIV seeder	High	Frequency and size regime closely overlap CRF6 design basis	Different device geometry
Ayati ~6 µm water droplets as gas-phase PIV tracer	High	Direct water-droplet tracer validation	Not an ultrasonic generator study
FDA 2026 warning letters	High regulatory relevance	Current enforcement language on dynamic smoke studies and neutrally buoyant media	Does not define a generation technology or numeric tracer specification
Auvinen 1.7 MHz / 6-8 µm thermal-plume counterexample	High limitation evidence	Shows why finished-plume thermal qualification is necessary	Different device, liquid formulation, and study objective

Appendix C. References

[1] U.S. Food and Drug Administration. Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice: Guidance for Industry. September/October 2004. FDA-2003-D-0145.

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

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

[4] U.S. Food and Drug Administration. Warning Letter: Sato Pharmaceutical Co., Ltd., MARCS-CMS 723059. May 18, 2026.

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

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

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[10] 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.

[11] Tsai CS, Mao RW, Tsai SC, et al. Faraday Waves-Based Integrated Ultrasonic Micro-Droplet Generator and Applications. Micromachines. 2017;8(2):56. doi:10.3390/mi8020056. Figure 9 used under CC BY 4.0.

Source-selection note: Regulatory claims are grounded in FDA primary sources. Scientific claims are grounded in peer-reviewed journal literature. Contrary evidence regarding thermal plume bias is included intentionally because a defensible regulatory position should identify and control known limitations rather than omit them.