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CALIBRATION AND VERIFICATION OF AN EXPOSURE SYSTEM FOR *YERSINIA PESTIS* CO92 IN A BSL-3 FACILITY

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Abstract

Objectives: For inhalation studies, bioaerosol instrumentation must be calibrated. The containment of such instrumentation in biosafety level 3 (BSL-3) laboratories presents researchers with challenges to maintain manufacturer's calibration specifications. Decontamination procedures can result in instrument damage, and some manufacturers will not accept instruments for recalibration once contaminated. Furthermore, most manufacturers lack personnel who are able to enter BSL-3 laboratories. Here, we describe our approach to verifying instrument calibration for studies aerosolizing *Yersinia pestis* CO92. Verification procedures were designed for a primary flow calibration device (BIOS DryCal® DC-Lite) (PFCD), gas flow controllers (Alicat Mass and Volumetric Precision Gas Flow Controllers) (GFC), and an aerosol monitor (TSI DustTrakTM Aerosol Monitor) (DT) used in BSL-3 containment. Additionally, the TSI Aerodynamic Particle Sizer® Spectrometer (APS) was calibrated in our BSL-3 laboratories.

Methods: The PFCD was verified for vacuum applications with custom designed critical orifi with volumetric flow rates from 1.0 to 20.3 and for pressure applications with a nebulizer. GFC flow rates were verified with the PFCD. Saline solutions were made to specific concentrations and aerosolized to verify the DT. APS flow rates were then calibrated with the PFCD. Polystyrene latex microspheres were used to calibrate the APS.

Results: The PFCD flow rate was verified over a flow rate range of 1.0 to 20.3 L/min. Observed flow rates were 2.4±1.6% of the factory calibrated specifications. The GFC flow rates were 1.9±1.4% of expected the values. Three concentrations of saline solution were used to determine the baseline aerosol concentrations for DT verification. The APS aerosol and sheath flow rates were calibrated to 1.0% and 0.3% of the recommended factory settings, respectively. The APS was calibrated over a range of particle sizes from 0.43 to 20.00 µm.

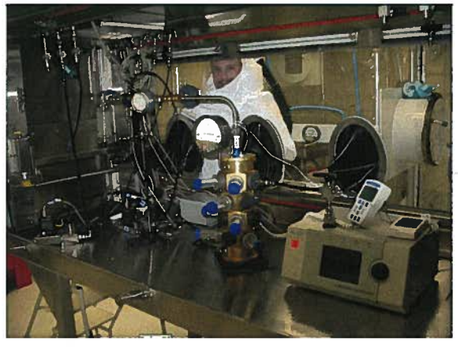
Conclusions: These studies demonstrate verification our BSL-3 bioaerosol instruments to be within the recommended calibration settings.

Introduction

Inhalation studies require an aerosol system comprised of multiple equipment networked to produce a quantitative bioaerosol. Since measurements taken to characterize and quantify the aerosol product must be precise, the equipment must be calibrated on a yearly basis according to the manufacturers specifications.

Inhalation systems located within a BSL-3 can be exposed to multiple infectious agents, including highly pathogenic organisms such as *Yersinia pestis* CO92, the causative agent of plague. In order to remove the equipment from the BSL-3, the equipment must undergo a decontamination process that may damage the equipment. Many manufacturers will not enter the BSL-3 facility and most manufacturers will not accept decontaminated equipment for calibration. Given these challenges, trained personnel and procedures must be put in place to maintain the equipment.

Figure 1. BSL-3 Aerosol Inhalation and Exposure System



We designed standard operating procedures in conjunction with the manufacturer's specifications and had personnel trained by the manufacturer to calibrate and verify the BSL-3 aerosol equipment. We present a calibrated system for use in challenge studies with *Yersinia pestis* CO92 that contains a primary flow calibration device (BIOS DryCal® DC-Lite) (PFCD), gas flow controllers (Alicat Mass and Volumetric Precision Gas Flow Controllers) (GFC), an aerosol monitor (TSI DustTrak™ Aerosol Monitor) (DT), and the TSI Aerodynamic Particle Sizer® Spectrometer (APS).

Methods

Calibration and validation methods:

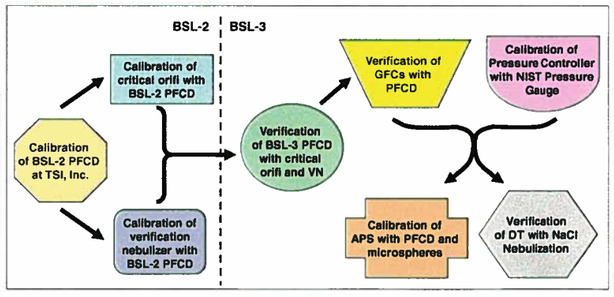
PFCD: The BSL-3 PFCD was verified for vacuum applications with custom designed critical orifi with volumetric flow rates from 1.0 to 20.3 and for pressure applications with a nebulizer to be within 10% of factory calibrated values. Critical orifi were measured using a factory calibrated PFCD in a BSL-2 area to obtain flow rates of 1.0 to 20.3 L/min. A single marked verification nebulizer (VN) was pressurized at 15, 20, 30, and 40 psig and readings were taken using the calibrated PFCD in a BSL-2 area. The calibrated critical orifi and the calibration nebulizer were transferred into the BSL-3, and then used to verify the BSL-3 PFCD.

GFCs: Each GFC flow rate was determined with the verified PFCD. The GFCs did not undergo calibration procedures since their set points were within 10% of factory calibrated values as stated by the SOP. Each GFC was independently set to five values that encompass its range after it was attached to a verified PFCD (Table 1). Note each GFC has a different range and therefore different calibration values. Each range value was read independently and averaged per range point.

APS: The APS was calibrated according to the training provided by TSI, Inc. For the APS, two parameters needed to be calibrated 1) the flow rate into the APS (aerosol and the sheath flow rates) and 2) the particle size that it detects. The flows were adjusted according to manufacturers guidelines. Then polystyrene microspheres with particle sizes of 0.43, 0.50, 0.67, 1.00, 4.967, 6.992, 10.030, 15.020, and 20.000 µm were used for the APS calibration.

DT: Three saline solutions were made to 0.010%, 0.045%, and 0.090% and aerosolized to verify that the DustTrakTM Aerosol Monitor, DT, read the same concentrations over time.

Figure 2. Schematic of Aerosol Instrumentation Verifications and Calibrations



Results

BIOS DryCal® DC-Lite

The PFCD determines the flow rate (L/min.) of instruments. Positive and negative pressure causes the movement of a dry piston in the PFCD. The rate of the movement is electronically converted into the flow reading. The PFCD controls samplers, air dilution devices, and exhaust devices.

Table 1. PFCD Verification

Verification Device ID	Calibration Flow Rate (L/min.)	Mean Flow Rate (L/min.)	Percent Difference (%)
VN1	16.314	15.83	2.95
VN1	13.182	12.87	2.37
VN1	10.174	9.90	2.73
VN1	8.457	8.26	2.29
CO 1.2	0.964	0.91	5.31
CO 5.1	5.103	5.17	1.27
CO12.1	11.606	11.62	0.09

(VN = verification nebulizer, CO = critical orifice)

Mass and Volumetric Precision Gas Flow Controllers

Table 2. GFC validation data

Serial Number	GFC flow rate (L/min.)	PFCD mean flow rate (L/min.)	Percent difference (%)
40902	3	2.93	2.4
40902	9	8.85	1.6
40902	15	14.72	1.9
40902	21	20.48	2.5
40902	25	25.37	1.5
40899	1	1.00	0.3
40899	3	3.01	0.4
40899	5	5.05	0.9
40899	7	7.11	1.6
40899	9	9.23	2.5
25073	1	1.00	0.0
25073	3	3.04	1.5
25073	5	5.14	2.7
25073	7	7.28	3.9
25073	9	9.49	5.4

The GFCs control the flow rates (L/min.) of the mixer, diluter, and chamber exhaust. A signal is sent to the proportional valve to adjust the diameter to deliver the desired flow rate.

Since the highest percent difference was 5.4% (Table 2), all the GFCs were within calibration.

Aerodynamic Particle Sizer® and Spectrometer

The APS determines the aerosol particle size and particulate distribution. Particles enter the APS pass through two laser beams and a detector to calculate the particle size and distribution.

The aerosol flow rate was calibrated to 1.010 L/min. and the sheath flow rate was calibrated to 3.990 L/min. The spheres, a TSI APS calibration program, and apsCalib.exe, were used to create a standard curve (Figure 3) and then used to update the APS calibration. Additionally, after the calibration was completed *Yersinia pestis* CO92 was run to verify size distribution, a preparation of 5E9 CFU in 1.0% peptone and 50% BHI was used (Figure 4).

Figure 3. APS Particle Calibration Curve

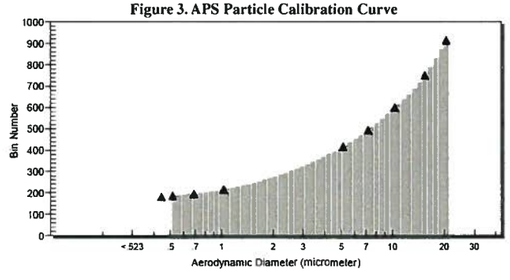
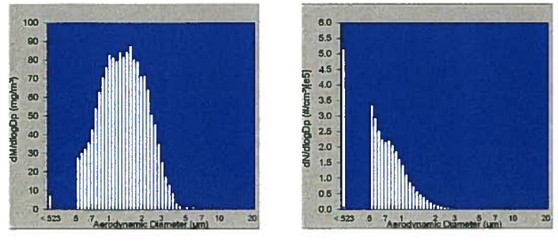


Figure 4. *Yersinia pestis* CO92 APS profiles



DustTrak™ Aerosol Monitor

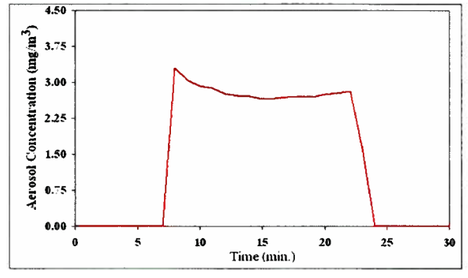
The DT determines the relative aerosol particle concentration that is present in the chamber. A vacuum pump creates a flow rate of 1.7 L/min. at the nozzle of the DT. The DT directly samples the exposure particles. The particles enter the DT and are counted by laser.

The DT flow rate was adjusted from 1.506 L/min to 1.704 L/min. Three different salt solutions (0.010%, 0.045%, and 0.090%) were nebulized three times each to verify that the relative concentration detected by the DT did not change. The values are listed below (Table 4). Additionally, post verification *Yersinia pestis* CO92 was sampled and a DT graph was generated (Figure 5).

Table 4. DustTrak validation data

Run Number	DustTrak Value for 0.010%	DustTrak Value for 0.045%	DustTrak Value for 0.090%
1	2.37	10.3	23.1
2	2.22	10.7	22.5
3	2.20	10.2	22.5
Mean	2.26	10.4	22.7
Stdev.	0.1	0.3	0.3
%CV	4.1	2.5	1.5

Figure 5. DustTrak of *Yersinia pestis* CO92



Conclusions

- **The BIOS DryCal® DC-Lite was verified to be within 10% of factory calibrated values.**
- **The Mass and Volumetric Precision Gas Flow Controllers were verified to be within 10% of factory calibrated values.**
- **The Aerodynamic Particle Sizer® and Spectrometer flows and particle size readings were calibrated to near factory settings.**
- **Also, the DustTrak™ Aerosol Monitor was shown to be within calibration.**
- **Both the APS and the DT produced expected results.**

Acknowledgements

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