SOUTHERN RESEARCH

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# Validation of a Rabbit and Non-Human Primate Inhalation Challenge System to Support FDA Licensure of Medical Countermeasures for Biothreat Agents

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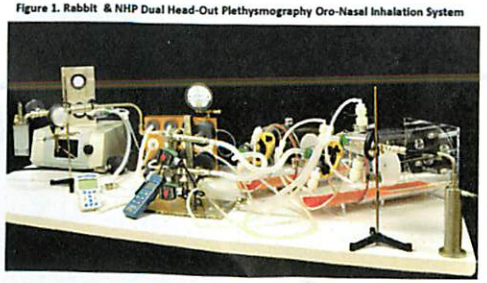
## Abstract

In order to support the licensure of medical countermeasure's against biodefense pathogens under the "Animal Rule" (21 CFR 314.600 & 21 CFR 601.90) an inhalation challenge system was developed and validated for use with rabbits and nonhuman primates. The major system components include the Aerosol Delivery Line consisting of a Collison Nebulizer, Passive Diluter and Radial Mixer, a 12-Port Inhalation Plenum, Electronically Actuated Solenoid Switching Platform, Challenge and Sampling Masks, Aerodynamic Particle Sizer (APS), Gas Flow and Pressure Controllers, Liquid Impingers, Oxygen Monitor, Humidity and Temperature Meter, and Head-Out Plethysmographs. Each of the components was selected so that the system could be appropriately configured to deliver respirable aerosols of bacterial spores, vegetative bacteria or viruses to the breathing zone of nonhuman primates or rabbits, in pairs or individually. User Requirements Specifications established for the system included system performance, security, audit trail, regulatory, interface, backup and recovery, and range and logic requirements. The system was installed in the Biosafety Level 3 laboratory and evaluated following approved Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) specifications to demonstrate that the system meets the User Requirement Specifications. No technical errors were identified during the IQ tests and the OQ tests demonstrated that the system would operate within acceptable environmental parameters and that excursions outside of those parameters were detectable. The OQ test also demonstrated that the system operated within an acceptable time to reach 90% of the target aerosol concentrations and the aerosols were delivered homogenously to each sampling and challenge mask of the system. The PQ tests demonstrated that appropriate temperature, relative humidity, and oxygen concentrations were achieved during actual operation of the system. No limitations to the system were observed during the validation of the Large Animal Inhalation Challenge system and it is approved for use in its current state to support studies under Good Laboratory Practice standards.

## Introduction

Under 21 CFR 314.600 and 21 CFR 601.90 new drug products and new biologics, respectively, can be granted marketing approval based on adequate and well-controlled animal studies when definitive human efficacy studies would be unethical and field trials after an accidental or hostile exposure are not feasible. Such is the case for many of the biodefense pathogens on the NIAID priority pathogens list. A key component to conducting well controlled animal studies with biodefense pathogens is the ability to effectively and reproducibly deliver uniform and respirable aerosols to the breathing zone of the test system. A dual head-out plethysmography oro-nasal inhalation system for use with rabbits and NHP's has been developed at Southern Research Institute (Figure 1) and the validation of the instrumentation to support GLP studies of new drug products and new biologics against biodefense pathogens is described.

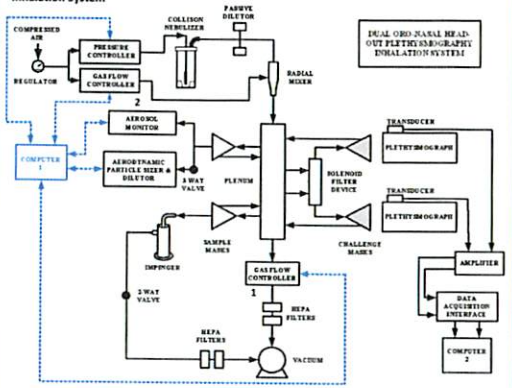
**Figure 1. Rabbit & NHP Dual Head-Out Plethysmography Oro-Nasal Inhalation System**



## Instrument Qualification

The inhalation challenge system, hereafter referred to as the system, was installed according to the schematic in Fig. 2 in a Class III Biosafety Cabinet within the animal BSL-3 laboratory of Southern Research Institutes Birmingham, AL campus. Installation was performed by personnel (testers) appropriately trained on handling, set up, operation, and maintenance of the equipment, instrumentation, and devices of the system. The testers documented each step of the installation according to the IQ Test Scripts which describe the system hardware, software, operating parameters, and environmental operating conditions.

**Figure 2. Design schematic of the Rabbit & NHP Dual Head-Out Plethysmography Oro-Nasal Inhalation System**




## Operational Qualifications

The two primary functions of the System are:

1. Provide physical and environmental support for the test system
2. Deliver a respirable aerosol, at a target concentration and particle size distribution, to the breathing zone of the test system.

Following successful IQ, the OQ testing strategy was to demonstrate the System will operate within acceptable environmental parameters for rabbits and NHP's and that excursions outside of the acceptable limits would be detectable; as well as to demonstrate the integrity of the System and its ability to efficiently deliver aerosols.

## Leak Test

The leak rate ($k$) of the System, including the plenum, challenge masks, and sample masks was determined by measuring the change in pressure over time in a sealed System.

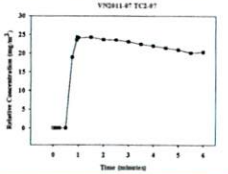
$k = \ln(\Delta P_1/\Delta P_2)/t_2 - t_1$ *if $k < 1.0$ than the leak rate is acceptable.

$k_{system} = 0.94$ $k_{masks} = 0.80$

## Environmental Specifications

| Specification | Expected result | Actual Result |
|---|---|---|
| Flow rates and System pressure (ΔP) with a computer | GFC 1 = 15 ± 1.5 L/min<br>GFC 2 = 5 ± 0.5 L/min<br>PC1 = 30 ± 3 psig<br>System pressure:<br>-0.1 ≤ ΔP ≤ 1.0 in. water | GFC 1 = 14.868 L/min<br>GFC 2 = 5.010 L/min<br>PC1 = 30.51 psig<br>ΔP = -0.4 |
| Flow rates and pressure control operation without a computer | GFC 1 = 15 ± 1.5 L/min<br>GFC 2 = 5 ± 0.5 L/min<br>GFC 3 = 1.5 ± 0.2 L/min<br>PC1 = 30 ± 3 psig | GFC 1 = 14.888 L/min<br>GFC 2 = 4.974 L/min<br>GFC 3 = 1.480 L/min<br>PC1 = 30.45 psig |
| Class III BSC operating pressure (ΔP) | -1.0 < ΔP ≤ -0.3 in. water | ΔP = -0.584 |
| Temperature (T) | 19°C ≤ T ≤ 25°C | T = 24.5°C |
| Relative Humidity (RH) | 30% ≤ RH ≤ 70% | RH = 37.4% |
| Oxygen concentration ($O_2$) | 18% ≤ $O_2$ ≤ 25% | $O_2$ = 20.9% |
| Oxygen ($O_2$) excursion | $O_2$ initial > $O_2$ final | $O_2$ initial = 20.9%<br>$O_2$ final = 15.0% |
| Temperature (T) excursion | T initial > T final | T initial = 22.5°C<br>T final = 22.0°C |
| Pressure (ΔP) excursion from compressed gas supply & correction | ΔP initial < ΔP disrupted<br>ΔP corrected = ΔP initial | ΔP initial = -0.4<br>ΔP disrupted = -0.6<br>ΔP corrected = -0.4 |
| Pressure (ΔP) excursion from vacuum supply & correction | ΔP initial < ΔP disrupted<br>ΔP corrected = ΔP initial | ΔP initial = -0.4<br>ΔP disrupted = 0.4<br>ΔP corrected = -0.4 |

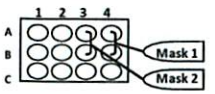
## Time to Achieve 90 % Target Concentration



- The $T_{90}$ in the mask was accessed using *B. anthracis* Ames spores.
- Settings: PC = 30 PSI, Exhaust GFC = 20 L/min., Mixer GFC 5 L/min
- The system requirement was set at $T_{90}$ < 5 min.
- The $T_{90}$ for the system was determined to be 0.789 min.

## Aerosol Concentration Homogeneity

| Test Performed | Location (column/row) | Sample Start Time (min.) | Relative Conc. (mg/m³) | Mean (mg/m³) | Stdev (mg/m³) | CV (%) |
|---|---|---|---|---|---|---|
| Total 1 | 3 / A,B | 7 | 6.85E+05 | 6.52E+05 | 1.33E+05 | 20.4 |
| Total 2 | 4 / A,B | 10 | 5.06E+05 | | | |
| Total 3 | 3 / A,B | 13 | 7.65E+05 | | | |
| Temporal 1 | 3 / A,B | 7 | 6.85E+05 | 7.97E+05 | 1.31E+05 | 16.4 |
| Temporal 2 | 3 / A,B | 16 | 7.65E+05 | | | |
| Temporal 3 | 3 / A,B | 25 | 9.41E+05 | | | |




Aerosol concentration homogeneity at the sample and challenge masks was determined by collecting spores from different plenum ports (total) or from one port at different times (temporal).

The port-port variability ($\%CV_{spatial}$) = $((CV^2_{total}) - CV^2_{temporal})^{1/2}$ = 12.1 %

## Controlling Aerosol Flow to Challenge Masks

**Figure 3. HEPA Filtered Solenoid Control Device**

- The ability of the solenoid to direct aerosols of *B. anthracis* Ames spores or clean air toward or away from the challenge masks separately was demonstrated.

## Stability of Aerosols at Sample Mask

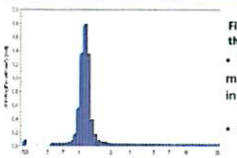
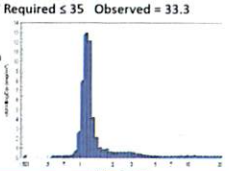


**Figure 4. Example of Aerosol Concentration in the System**

- The aerosol concentration at the sample mask during three independent tests was in the acceptable range.
- %CV Required ≤ 35 Observed = 33.3

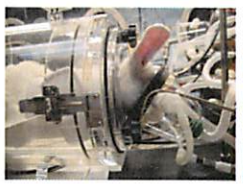
**Figure5. Example of the Particle Size Distribution in the System**

- The particle size distribution at the sample mask during three independent tests was in the acceptable range.
- GSD <2.5 Observed = 2.2, 2.1, 2.2



## Performance Qualification

For the PQ all environmental and aerosol requirements outlined in the OQ were confirmed while NHP's and rabbits were on the System.



## Conclusions

- The System is highly configurable and was validated in the most complex state.
- There were no observed limitations to the System in this configuration.
- Changes to the system such as equipment, organism or animal species may require verification in the pertinent IQ/OQ/PQ Test Scripts.
- The System passed all testing and is ready to be used for GLP studies.

## Acknowledgements

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