Volume 1
Number HE04

# The Whitley Internal HEPA filtration system – bacteriological testing

Andrew Pridmore
July 2014

## Introduction

As described in Technical Note HE03, Whitley Workstations equipped with our Whitley Internal HEPA Filtration System achieve particle counts in the internal atmosphere that exceed the requirements of ISO 14644-1 Class 3. To our knowledge, no other manufacturers of positive pressure modified atmosphere workstations can approach anything like compliance with the stringent standards we attain. Please review other DWS Technical Notes in this series for a complete understanding of the superiority of the Whitley Internal HEPA Filtration System.

Due to the types of the work performed by users of our hypoxia workstations and anaerobic workstations, we consider it important to augment the particle count data with specific proof that the HEPA filtration system is capable of removing bacteria from the atmosphere. Therefore, we conducted further laboratory tests to challenge the atmosphere with high concentrations of aerosolized bacterial cells and spores and to quantify their removal.

## Test Method

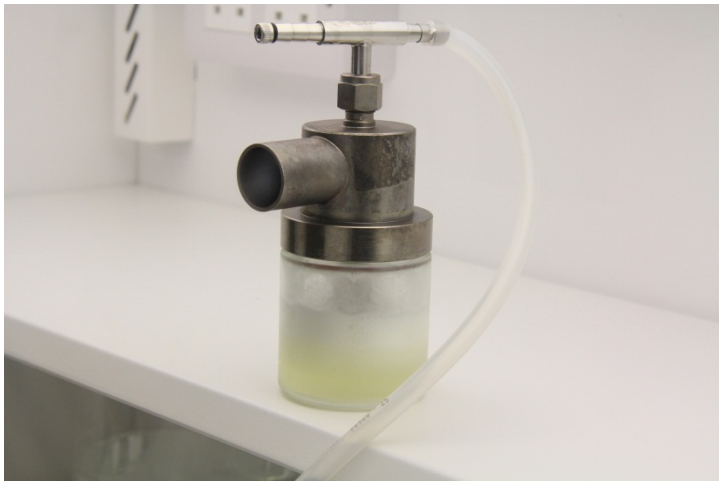
An H35 HEPA and an H45 HEPA hypoxystation were tested. In addition, a standard H35 hypoxystation (no HEPA) was tested to provide comparative data. Bacterial strains used for the test were the non sporeforming aerobe *Kocuria rhizophila* and the sporeforming anaerobe *Clostridium beijerinckii* (both with low pathogenic potential). To produce a suspension of vegetative cells, *K. rhizophila* was subcultured aerobically on Tryptone Soy Agar (TSA) at 37°C for 2 days. Cells were harvested into sterile Maximum Recovery Diluent.

To produce a spore suspension, *C. beijerinckii* was cultured anaerobically in 2 × 500 ml volumes of Bryant and Burkey medium at 37°C for 10 days. The two liquid cultures were heated at 80°C for 10 minutes, centrifuged to pellet the spores and cell debris, washed 3 times in sterile 0.85% NaCl and the combined material from both cultures was resuspended in 100 ml of sterile 0.85% NaCl to concentrate the spores.

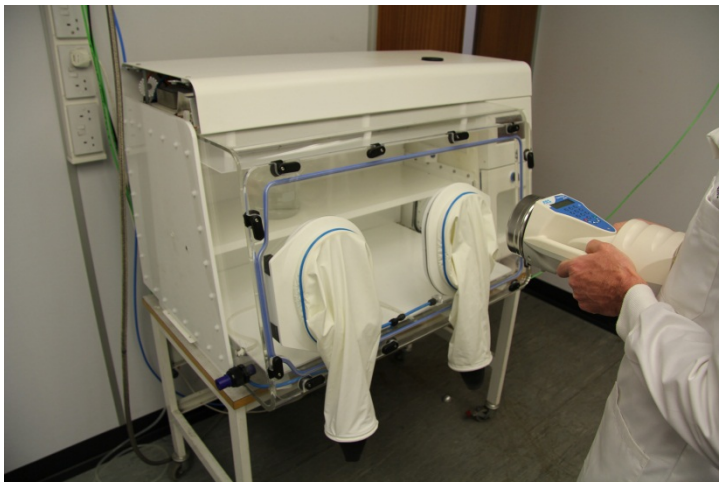
Viable counts (determined by plating) were $>1\times10^8$ cfu/ml for *K. rhizophila* cell suspension and $>1\times10^5$ cfu/ml for *C. beijerinckii* spore suspension.

Before testing, each workstation was allowed to stabilize at 37°C and 75% relative humidity and was operated continuously throughout the test so that normal atmospheric circulation occurred. A 55 ml volume of *K. rhizophila* cell suspension or *C. beijerinckii* spore suspension was added to a 6 jet NSF Collison nebulizer (BGI Inc; http://www.bgiusa.com) and the fully assembled nebulizer was weighed.

The nebulizer was transferred into the workstation, placed on the upper shelf with the outlet facing the front of the chamber and connected to compressed air at 20 psi pressure and 12 litres per minute flow rate. Nebulization was performed for 5 minutes, after which the nebulizer was weighed again to determine the dispensed volume.



Sampling of the internal workstation atmosphere was conducted using an AES Sampl'air Lite (bioMérieux). Samples of 100 litres were collected by impaction onto a 90 mm petri dish of TSA (*K. rhizophila* experiments) or Fastidious Anaerobe Agar (FAA; *C. beijerinckii* experiments). The atmosphere was sampled during the final 1 minute of nebulization, immediately after completion of nebulization and at timed intervals during the subsequent 30 minutes.



## Results

Bacterial colony count data obtained from the air sampler plates are tabulated below (note that the upper detection limit for the air sampler is 258 colonies). The standard H35 workstation allowed *K. rhizophila* to persist, without a measurable reduction in viable count, for at least 30 minutes after nebulization (Table 1). An additional sample after 4 hours also revealed high numbers of the organism (not shown in the table).

In contrast, *K. rhizophila* count in the atmosphere of the H35 HEPA hypoxystation 2 minutes after nebulization was reduced more than 100-fold in comparison with the standard H35 and the organism could not be recovered from the H35 HEPA after 5 minutes (Table 2). Results obtained in the H45 HEPA were similar (Table 3). On the basis of these results, the performance of H35 / H45 HEPA hypoxystations exceed the requirement stipulated in ISO 14644 that the appropriate class of atmospheric cleanliness should be regained within 20 minutes following any disruption. This is in agreement with the particle count data reported in Technical Note HE03.

To test the capacity of the HEPA filter to remove bacterial spores, nebulization experiments were conducted in the H35 HEPA using *C. beijerinckii* spores. Sampling of the internal atmosphere after nebulization of the spore suspension produced a small colony count immediately after the nebulizer was stopped, then no recovery of the organism after 2 minutes. Thus, the HEPA filter was also highly effective in the removal of spores from the atmosphere. The shorter time required to achieve zero counts, in comparison with the *K. rhizophila* results, was probably attributable to the lower viable count attained in the spore suspension.

## Conclusions

Our experiments have demonstrated that the HEPA filtration system in Whitley workstations achieves a rapid and substantial reduction in bacterial contamination of the atmosphere. It should be noted that these results are equally applicable to the equivalent models of anaerobic workstation (A35 HEPA) because their HEPA filtration systems are identical to those in the equivalent model of "H" workstation.

## Results

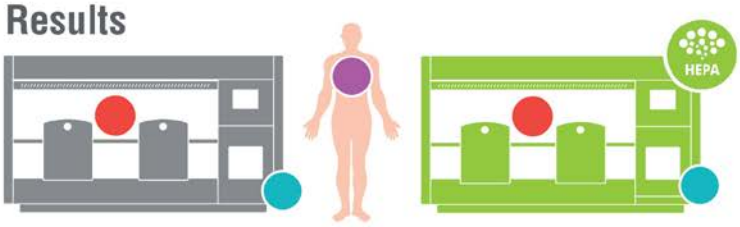



**Table 1:** Recovery of *K. rhizophila* colonies from standard anaerobic chamber (No HEPA)

| Before nebulization | During nebulization ($1.9x10^8$ cfu / 5 min) | After nebulization | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| 0 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 |
| 0 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 |
| Before nebulization | During nebulization ($1.2x10^8$ cfu / 5 min) | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| | | After nebulization | | | | | | |

**Table 2:** Recovery of *K. rhizophila* colonies from HEPA filtered anaerobic chamber

| Before nebulization | During nebulization ($2.1x10^8$ cfu / 5 min) | After nebulization | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| 0 | ≥258 | 55 | 2 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Before nebulization | During nebulization ($1.2x10^8$ cfu / 5 min) | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| | | After nebulization | | | | | | |

**Table 3:** Recovery of *C. beijerinckii* colonies from HEPA filtered anaerobic chamber

| Before nebulization | During nebulization ($3.3x10^5$ cfu / 5 min) | After nebulization | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| 0 | 232 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 4:** Recovery of *K. rhizophila* colonies from operator's position (No HEPA)

| Before nebulization | During nebulization ($1.9x10^8$ cfu / 5 min) | After nebulization | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| 0 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 | ≥258 |

**Table 5:** Recovery of *K. rhizophila* colonies from operator's position (HEPA)

| Before nebulization | During nebulization ($1.2x10^8$ cfu / 5 min) | After nebulization | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 min | 5 min | 10 min | 15 min | 20 min | 30 min |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

*Numbers of colonies recovered are all from 100 litre air samples*
*Upper detection limit for air sampler = 258 colonies*

This work was presented at Anaerobe 2014, Chicago, USA. A copy of the poster is available to download from www.dwscientific.co.uk.

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