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HEPA filtration of gas discharged from Whitley workstations: bacteriological testing

Pridmore A, November 2014

Abstract

Some users of HEPA filtered Whitley anaerobic workstations and Hypoxystations require additional precautions to reduce the possibility of bacteria being discharged from the chamber via the gas outlets. The two gas outlets present on Whitley "A" and "H" workstations are the main chamber exhaust valve and the interlock exhaust valve. The HEPA versions of our workstations can now be specified with optional HEPA filter canisters fitted to the exhaust valves (these filters cannot be specified for non-HEPA workstations). We have conducted laboratory tests to challenge the exhaust filters with high concentrations of aerosolized bacterial cells and quantify their discharge through the exhaust valve filters.

Test Method

Whitley H35 Hypoxystation

For the purposes of this test, an H35 Hypoxystation without internal HEPA filtration was used. Internal HEPA filtration was not used because its efficiency is such that aerosolized bacteria within the chamber are rapidly removed and a relatively low number would be available for discharge via the gas outlets. The workstation software was temporarily modified so that the interlock exhaust valve could be commanded to open while the inner interlock door was also open. Thus, the chamber atmosphere could be discharged through the interlock exhaust valve when required.

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The bacterial strain used for aerosolization within the chamber was the non spore forming aerobe *Kocuria rhizophila*, which has low pathogenic potential. This organism 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 cell suspension with a viable count (determined by plating) of 1.4×10^8 cfu/ml.

Before testing, the 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 was added to a 6 jet NSF Collison nebulizer (BGI Inc; http://www.bgiusa.com) and the assembled nebulizer was weighed.

6 jet NSF Collison nebulizer

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 6 minutes with the inner interlock door of the workstation left open, so that aerosolized bacteria could enter the interlock. On completion, the nebulizer was weighed again to determine the dispensed volume.

During nebulization, excess gas was discharged from the main chamber exhaust valve (via the HEPA filter canister) at 12 litres per minute (equal to the supply rate). During the 5th minute of nebulization, discharged gas was collected using an AES Sampl'air Lite (bioMérieux). The air sampler was held adjacent to the HEPA filter outlet and sampling was performed for 1 minute by impaction onto a 90 mm petri dish of TSA. During the 6th minute of nebulization, gas discharge was switched to the interlock exhaust valve and sampling of that valve's HEPA filter outlet was performed for 1 minute in the same manner.

Sampling of gas discharged through the HEPA filters of the Main chamber exhaust valve and interlock exhaust valve was repeated at 5 minutes and 10 minutes after nebulization was completed.

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Results

Bacterial colony count data obtained from the air sampler plates are tabulated below. No colonies were recovered during nebulization. After nebulization, the maximum number of *K. rhizophila* colonies subsequently recovered in one minute was 15. Based on the mass of bacterial suspension nebulized, 3.8×10^8 cfu of *K. rhizophila* were aerosolized into the chamber atmosphere during 6 minutes. Assuming that the gas sampled from each exhaust valve in a 1 minute period included the full 12 litres of discharged chamber atmosphere, then the number of cfu released in the absence of filtration would be 1.4×10^7 cfu. Thus, recovery of 15 colonies represents a 99.99989% reduction in bacterial density.

Conclusions

Our experiments have demonstrated that the HEPA filter cartridges fitted to the main chamber exhaust valve and the interlock exhaust valve of the H35 Hypoxystation achieve a substantial reduction in bacterial loading (better than 99.999%) within the discharged gas. It can be calculated that, if the number of bacteria released into the chamber atmosphere had been 1×10^7 cfu or lower, no bacteria would have been recovered from the exhaust gas discharged via the HEPA filters.

A bacterial load of 1×10^7 cfu is substantially higher than that which might be produced by any normal activities within an anaerobic or hypoxic workstation. Furthermore, bacteria released into the atmosphere of a Whitley workstation with internal HEPA filtration are rapidly removed (see Technical Note HE04). On this basis, the additional HEPA filter canisters fitted to the exhaust valves of HEPA filtered Whitley workstations offer a highly reliable degree of additional containment for any bacteria that might be present in the chamber atmosphere.

Based on the very satisfactory nature of these results, Don Whitley Scientific has introduced the Whitley Enhanced Biological Containment System.

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Table 1 Recovery of *K. rhizophila* colonies from HEPA-filtered gas outlets of the H35 workstation

Time point	Sampling location	*K. rhizophila* colony count on air sampler plate
Before nebulization	Main chamber exhaust	0
	Interlock exhaust	0
During nebulization	Main chamber exhaust	0
	Interlock exhaust	0
5 minutes after nebulization	Main chamber exhaust	3
	Interlock exhaust	4
10 minutes after nebulization	Main chamber exhaust	15
	Interlock exhaust	5

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