Protocol for the Validation of a Gas Decontamination process for Biological Safety Cabinets

Introduction
At the writing of this protocol, the use of formaldehyde gas has been the only process considered validated for gas decontamination of biological safety cabinets. Among other advantages, formaldehyde gas has been shown to meet two critical standards. First, being a gas at standard laboratory conditions, it is fairly easy to demonstrate that it can be circulated to all regions within sealed cabinets. Secondly, it demonstrates the ability to kill or make inactive bacterial spores when used under conditions as specified within NSF/ANSI 49 – 2007. More specifically, it has been generally shown to produce a 6-log reduction of the spore species Bacillus atrophaeus (formerly referred to as B. subtilis var. niger), typically used as a biological indicator for formaldehyde.

There are some disadvantages to the use of formaldehyde gas for this procedure. Formaldehyde is considered a carcinogen or potential carcinogen within much of the technical community. It is not currently registered as a gas-phase decontaminant by the US Environmental Protection Agency. A significant inconvenience is that typical formaldehyde use in a BSC leaves a residue consisting largely of paraformaldehyde on surfaces within the cabinet, which while removable from accessible surfaces, cannot be fully removed following the decontamination. As a result of such issues, alternative decontamination methodologies have been sought.

The NSF has decided that validation of an alternative decontamination system to formaldehyde gas should demonstrate that it is at least as effective as formaldehyde gas. Unfortunately, while formaldehyde has been the standard for decontamination for decades, no formerly recorded “full” validation study of this gas exists by which new methods may be compared. The following protocol was designed to fill this need. To demonstrate efficacy, it relies on the use of the same bacterial endospore, B. atrophaeus, as a biological indicator, targeting demonstration of a 6-log viable population reduction. To demonstrate appropriate penetrability to all interior parts of all types of Class II BSCs, the studies involve placement of BIs within the most challenging parts of the cabinet, and both type A and B cabinets are included in the study.

Protocol
1. Cabinet preparation
   a. The study shall include at least two different makes each of Class II Type cabinet, type A2 (both bench and console (console can be type A1) models), type B1 and type B2 biological safety cabinets. Each cabinet shall be decontaminated by the following procedures a minimum of three times.
   b. HEPA filters within the tested cabinets will have been previously “loaded” to an increase of greater than 0.3”w.g. (50%) of their starting clean value.
   c. Typically biological indicators consisting of $10^6$ B. atrophaeus endospores will be used for the validation study. Alternative indicator types might be used with approval of the NSF. The bacteria species, substrate and order of
magnitude of spore population shall be specified. The material of the BI
envelope, if any, shall also be specified.

d. Place a minimum of six pairs of appropriate biological indicators within the
biological safety cabinet (BSC). Locations include where possible:
   i. One pair of BIs is placed between the pleats on the downstream (clean)
side of the exhaust HEPA filter near the center. Two more pairs of BIs
are at opposite corners of the filter, placed between the pleats no more
than three inches from the nearest outside corner of the exhaust HEPA
filter.
   ii. One pair of BIs is placed within a potentially contaminated positive
pressure plenum.
   iii. One pair of BIs is placed beneath the work surface in the plenum
below the cabinet work area.
   iv. One pair of BIs is placed between the pleats near the center of the
upstream (dirty) side of the down flow HEPA filter.

e. Prepare the BSC for the decontamination process.

f. Provide a means, either within or external to the BSC, by which the air within
the BSC may be environmentally monitored throughout the decontamination
process.

g. Seal the BSC at the opening to the workspace and at or above the exhaust
port. Verify the adequacy of the seal.

2. Decontamination procedure

a. The methodology for the decontamination procedure during the validation
study must be clearly specified prior to the study. Several points should be
taken in consideration.
   i. If possible, the procedure should not designate the use of equipment
provided by unique manufacturers.
   ii. As the finally approved procedure may have a safety margin applied, it
would be useful to have the validation procedure designed at lower
chemical exposure (concentration and/or time) than what is intended
for ultimate field usage.
   iii. Generally, a decontamination method might involve either the
introduction of a calculated mass of decontaminant, dependent upon
the volume of the BSC, or the introduction of a gas whose
concentration is monitored and maintained during the
decontamination. The protocol should clearly state if either or both of
these methods are being validated.
   iv. The decontamination method may have requirements of permitted
humidity and/or temperature range within and outside of the BSC. The
protocol should clearly specify these and if there is a requirement that
such condition exists for a specific duration prior to the introduction of
decontaminant.
   v. The method should state clearly what events are to designate the
commencement and conclusion of the exposure period.

b. If at any time during the decontamination process the decontaminant is
detected in the environment exterior to the BSC by instrument or odor at a
concentration approaching its TEV level, stop generation of the decontaminate and do not resume until the leak source(s) has been corrected and it is safe to do so.

c. When the decontaminate is to be introduced as a fixed mass for the purpose of the validation study, it will be used at no more than 0.1 gram per cubic foot of cabinet volume for an 80-minute exposure.

d. In order to ensure a uniform concentration of the decontaminant throughout the BSC it may be advantageous to periodically operate the BSC’s internal blower (bump the BSC).

e. Monitor the environmental conditions at regular intervals during the decontamination process. Automatic continuous monitoring may be employed.

3. Scrubbing /venting

a. Determine if any scrubbing or venting is necessary to remove or render harmless the decontaminant used.

b. In order to ensure a full removal of the decontaminant from throughout the BSC it may be advantageous to periodically operate the BSC’s internal blower, a blower supplied with the scrubber, or both.

c. When the concentration of the decontaminant throughout the BSC is at the corresponding NIOSH STEL limit, and preferably below that, the BSC may be unsealed and vented.

d. Ensure minimal operation of the BSC blower(s) during the venting procedure to minimize the opportunity for contaminating the spore strips with airborne contaminants.

4. Analysis

a. Collect biological indicators

b. Have go/ no-go analysis (3-7 days) performed for surviving spores on strips, with the use of positive controls. **Effort should be made to reduce potential decontaminant residuals from BIs prior to placing them within growth media.**

c. The result at a site of a single trial will be deemed successful if either 1 or 2 BI’s from that site test negative (no turbidity in the incubated media tube.) If both strips test positive, that site test will be deemed a failure.

d. For a single cabinet trial, the trial would be considered successful (a pass) if all 6 site tests are successful by the criteria given above. It would be considered unsuccessful (a failure) if the site tests failed at more than 1 location. The trial would be considered a conditional pass if there was a failure at only one site.

e. A cabinet study is considered to have passed if all three trials passed. A cabinet study will also have been considered to pass if there had been one or more trials with conditional passes, as long as there has not been more than one failure for a given site.

f. A cabinet trial may be repeated if there is a clear understanding of the reason of a trial failure that is not based upon the intended target decontamination conditions. As examples, such reasons may include unexpected cabinet leakage, incorrect humidity levels or errors in BI handling.