*RESEARCH ARTICLE*

# Design, Development and Qualification of a Microbiological Challenge Facility to Assess the Effectiveness of BFS Aseptic Processing

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*ABSTRACT:* A programme of work has been initiated to further the understanding of the impact of the environment surrounding a Blow/Fill/Seal (BFS) machine upon the microbiological quality of processed product. A dedicated facility (Challenge Room) has been constructed and qualified to provide for the production and containment of dispersions of micro organisms in air of a room housing an operating BFS machine of a given style and configuration. The facility achieves homogeneous distribution of generated dispersions throughout the Challenge Room air, including that within and close to the critical area in which aseptic BFS operations occur. Generated microbial dispersions can be maintained in the room over extended time periods (up to 600 min) at a desired concentration within the range $10^1$ to $10^7$ cfu $m^{-3}$. They can also be produced employing different cell types, including bacterial endospores, Gram-positive and Gram-negative vegetative cells and yeast cells. Effective containment of dispersions is achieved while 'cards of product' (vials in sets) are conveyed from the Challenge Room to an adjacent Packing and Storage Area. Decontamination of the room and the housed BFS machine is accomplished through exposure to chlorine dioxide gas at a concentration of 1.0 mg $dm^{-3}$ for 120 min at room temperature (~23 °C).

*KEYWORDS:* Blow/Fill/Seal, BFS, microbial dispersions, containment, microbial challenges, chlorine dioxide decontamination

## Introduction

The merits of conducting media fills, in combination with controlled microbiological challenges, for providing evidence of the effectiveness of Blow/Fill/Seal (BFS) aseptic processing have been fully and unequivocally demonstrated in our previous studies. These have been of two main types:

(i) Challenges in which polymer granulate, uniformly contaminated with dry heat resistant spores, was processed to assess the lethality of the moulding of the container (1).

(ii) Challenges in which the BFS machine environment was loaded with air dispersed bacterial spores at various known levels ranging from $10^3$ to $10^7$ spores $m^{-3}$ to ascertain the assurance of sterility achieved with BFS processing. These challenges were carried out on a pre-production machine housed in a temporary containment laboratory that was located on a non-production site (2, 3).

To further develop the airborne challenge approach, a permanent, dedicated challenge facility is required capable of housing BFS machines of various types and configurations. Basic design requirements for such a facility are (a) possessing all services necessary to conduct media fill studies, (b) ensuring uniform dispersal of viable microorganisms at desired concentrations throughout the contained environment surrounding the operating machine, (c) maintaining the airborne microbiological challenge over extended periods of time, (d) having the ability to disperse and maintain in air test microorganisms of different aerodynamic sizes, (e) conveyance of media filled units processed within the contained environment to an ap-

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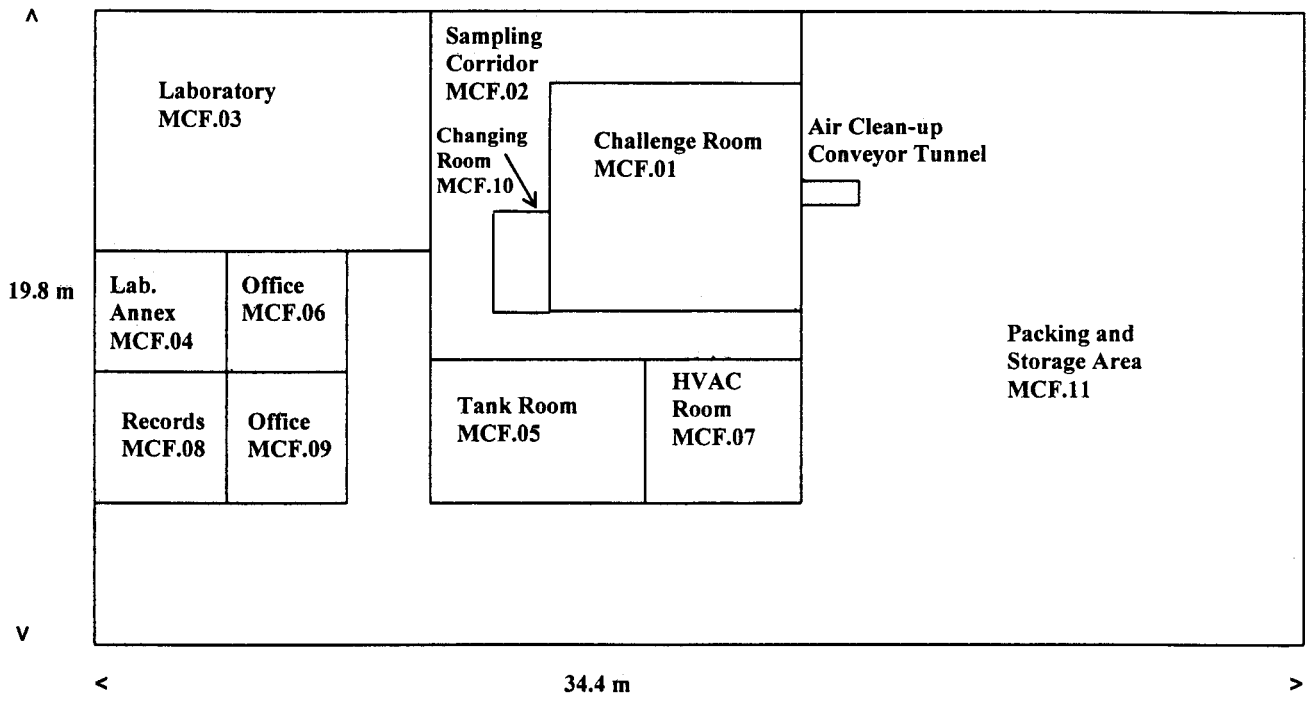


**Figure 1**

**Plan view of Microbiological Challenge Facility**

propriate packing area, and (f) permitting *in situ* decontamination of the challenge environment and the contained machine.

In addition to addressing sterility assurance issues specific to existing BFS machine configurations and operating conditions, such a facility could be employed as a 'test bed' for seeking optimal designs for new models of machines and their components.

This paper describes a permanent Microbiological Challenge Facility (MCF) built at Cardinal Health, Woodstock, IL primarily to understand better and assess the effectiveness of BFS aseptic processing. It also details an experimental qualification programme carried out to demonstrate that the facility possesses the critical design features identified above.

## The Microbiological Challenge Facility (MCF)

Figure 1 shows an overhead view of the layout of the MCF, which has an overall footprint of 19.8 by 34.4 m. The MCF comprises a number of interrelated rooms and services at the centre of which is the Challenge Room (designated MCF.01).

*Challenge Room*

The Challenge Room, of internal dimensions of 6.1, 7.3 and 3.4 m in width, length and height respectively, is comparable in size to rooms used for commercial production. The walls and ceiling are constructed from modular double-skin sandwich panels with foamed-in eccentric locking systems. The locking systems squeeze together the panels and intercalated neoprene gaskets, thereby forming airtight seals. The floor of the Challenge Room is covered with a homogenous solid vinyl with seams welded to provide a continuous surface. The Challenge Room is equipped with eight double-glazed cleanroom windows and six light fittings contained in flush leak-proof housings mounted in the ceiling. Of volume 151 $m^3$, the room is able to house readily either an ASEP-TECH® (Weiler Engineering, Elgin, IL) Model 624 or 640 style BFS machine. The installation of a BFS machine in the Challenge Room is achieved through removal of the sealed demountable panels that constitute part of the wall adjacent to the Packing and Storage Area (MCF.11). Following assembly, the room was confirmed to be 'leak-free' through integrity testing involving release of N-amyl acetate ("Banana Oil") in the Challenge Room, followed by olfactory examination outside the

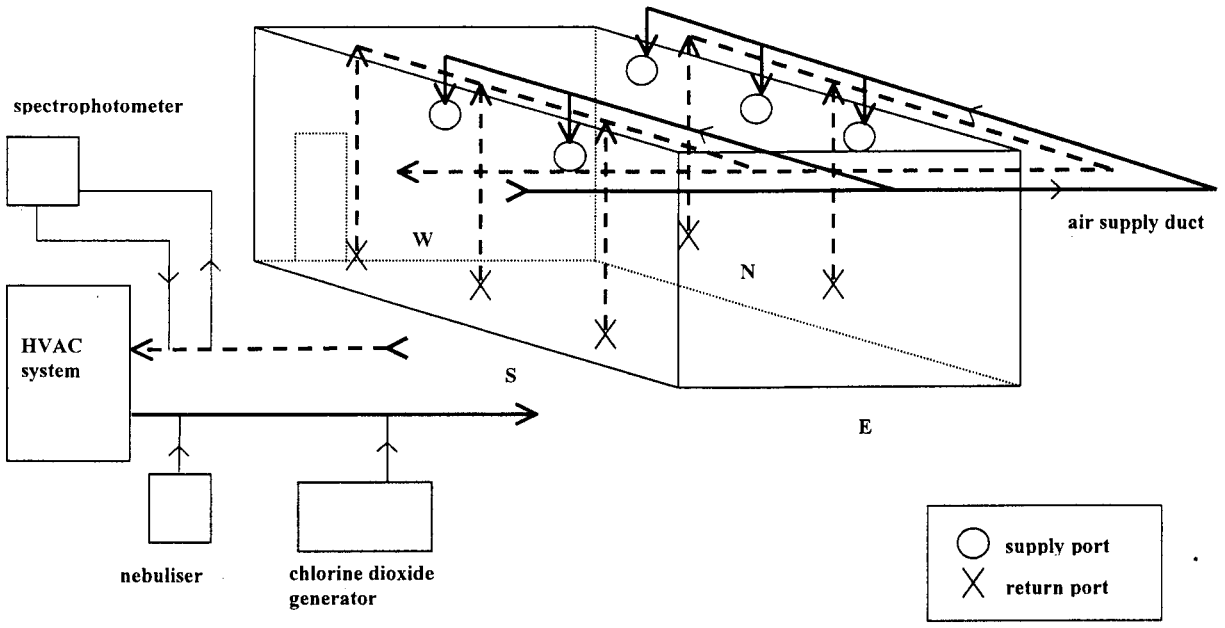


**Figure 2**

**Schematic representation of the handling of the circulating air of the Challenge Room**

room of all joints. Access to the machine sited in the room is gained through an airlock/changing room (MCF.10) located in the Sampling Corridor.

The Challenge Room is supplied with services necessary for machine operation and media fill studies. These services include electrical power, saturated steam, vacuum, compressed air, cooling water, polymer granulate supply and a line for delivery of filtered liquid media, all sealed into the floor or walls of the room.

*Air Handling and Production of Air Dispersed Microorganisms*

The air of the Challenge Room is re-circulated through what is essentially a 'closed-loop' controlled by a dedicated HVAC system located in MCF.07. The system is equipped with a 2 HP blower (rated at 2400 cfm) with variable frequency drive capable of delivering up to 68 $m^3$ $min^{-1}$ of air to the Challenge Room. Figure 2 is a schematic representation of the loop and of points of access for nebuliser input and chlorine dioxide input and sampling. Circulating air enters the Challenge Room via five high level, wall mounted, 25 cm diameter ports connected to a branched stainless steel supply duct; three ports are equally spaced on the North wall of the Challenge Room and two on the South wall. Air is extracted from the room through five low level 25 cm diameter ports connected to a common stainless steel return duct; two return ports are located in the North wall and three in the South wall. Since the purpose of the system is to introduce and maintain a pre-defined level of microorganisms in the air, there are no filters, HEPA or otherwise, located in the system. Re-circulated air is conditioned within the HVAC system employing copper heating and cooling coils that are coated with a polyelastomer to prevent corrosion. The HVAC system also provides for humidification/dehumidification. All welded seams of the ductwork were found to be 'leak-free' when bubble tested at 7.5 cm water pressure with dilute solution of surfactant. Smoke testing of the air movement within the Challenge Room demonstrated balanced flows with no stationary areas.

A nebuliser, described in U.S. Patent 5,301,878, is used to produce dispersions of challenge microorganisms in air from aqueous suspensions. In operation, the nebuliser provides droplets of relatively small size (5 micron diameter) containing discrete cells. Such small aqueous droplets, on expansion into air, evaporate to leave discrete cells suspended in air. The nebuliser possesses a reservoir volume of up to 300 $cm^3$ and, during continuous operation replenishment of the microbial suspension is achieved through transfer of

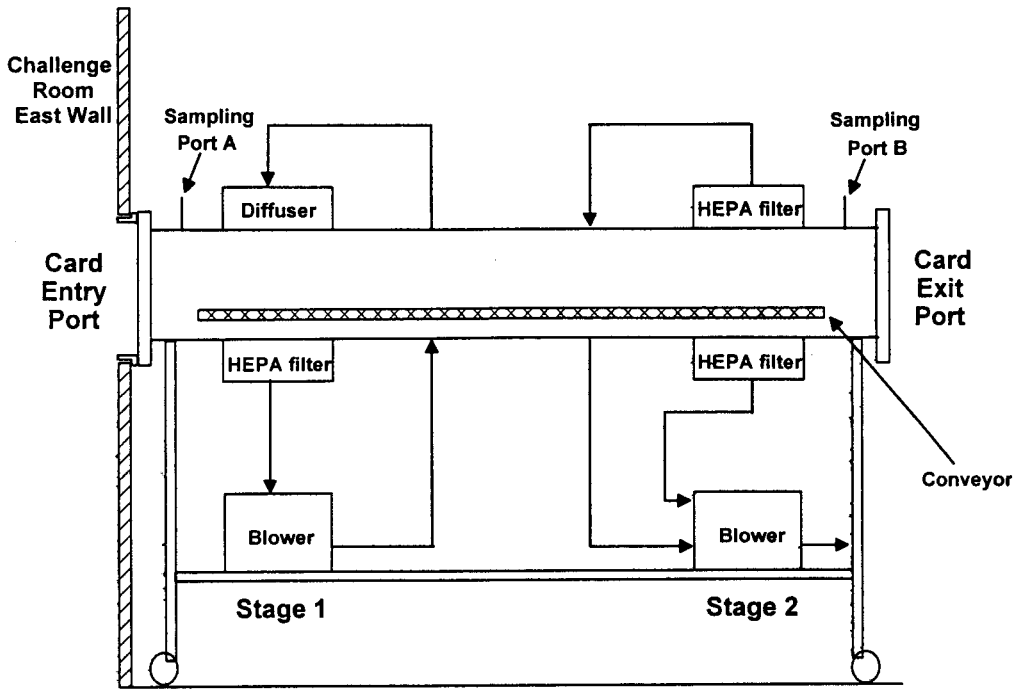


**Figure 3**

**Side view of Air Clean-up Conveyor Tunnel (ACCT)**

additional suspension from an adjacent recharge vessel. For ease of access, the nebuliser and ancillaries are located in the HVAC Room and the generated microbial dispersion is fed into the air circulating through the HVAC at the supply side of the blower. Re-circulation of the Challenge Room air acts to distribute dispersed microorganisms uniformly throughout the room.

*Conveyance of Filled Vials Exiting the Challenge Room*

Challenge experiments are envisaged in which an operating BFS machine, housed in the Challenge Room, will be executing a media fill in the presence of a known and controlled number of specified airborne microorganisms dispersed throughout the room. The outcome of such a media fill is a large quantity of processed vials containing growth medium; the vials are produced as 'cards'; a card comprises a set number of vials (up to 30, depending on the size of the vials) formed during a single BFS cycle. In order to label, package, incubate and ultimately 'read' the media fill vials, the cards have to be transferred from the Challenge Room to an adjacent Packing and Storage Area; this transfer has to be effected without the transmission of air dispersed microorganisms. To achieve this, processed cards must be separated from the contained dispersion without hindering them exiting the Challenge Room and compromising the contained dispersion. Such conveyance has necessitated the design and qualification of an Air Clean-up Conveyor Tunnel (ACCT).

The ACCT (see schematic representation in Figure 3) comprises a 3.5 m rectangular section fabricated from sheet metal with an open face of 31 by 45 cm. The entry port of the ACCT is connected and sealed to an appropriately sized opening in the East wall of the Challenge Room. A centrally located, electrically driven belt conveys BFS cards through the tunnel. To prevent air dispersed microorganisms passing through the tunnel during card conveyance, the ACCT provides a two stage air clean-up process, with each stage having an independent blower system. Stage 1, adjacent to the entry port, is effectively an air wash/curtain operating to recirculate air entering the tunnel through a HEPA filter and a diffuser located on the lower and upper surfaces of the tunnel, respectively. Stage 2, adjacent to the card exit port, operates to draw air from the tunnel and to expel the drawn air to atmosphere through separate HEPA filters located on the tunnel's upper and lower surfaces. In practice, operation of the Stage 2 blower induces a uniform airflow into the

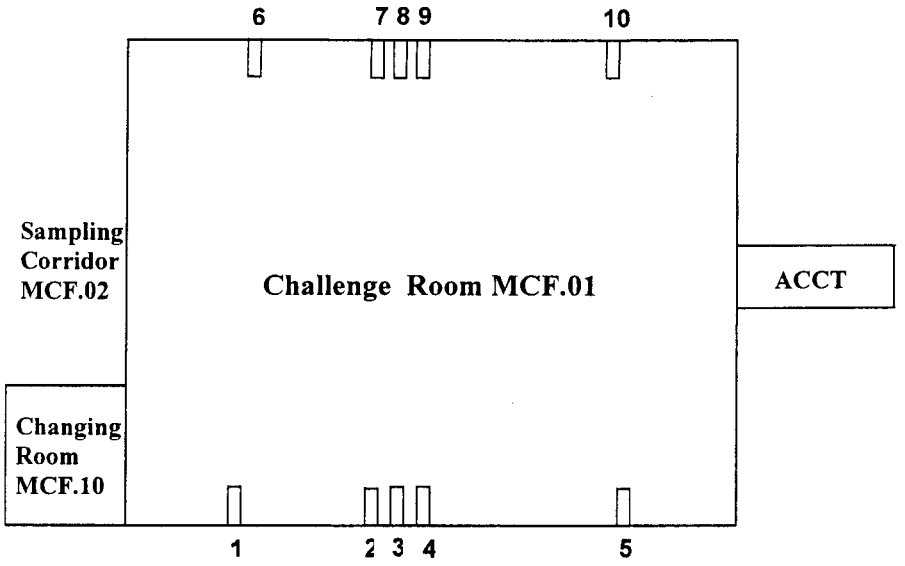


**Figure 4**

**Plan view of locations of air sampling ports**

tunnel across the entire face of the exit port. Air flows at the two stages of the ACCT can be adjusted independently through appropriate changes in fan speeds and are set to achieve minimally (a) a volumetric flow of 5 $m^3$ $min^{-1}$ at Stage 1, and (b) an air velocity into the tunnel at the exit port of 0.5 m $s^{-1}$.

To allow assessment of the effectiveness of the ACCT in preventing release of dispersed microorganisms from the Challenge Room to the Packing and Storage Area, the ACCT provides two air sampling ports, designated A and B. These ports permit air volumes to be drawn from the tunnel before and after the two clean-up stages.

*Air Sampling System*

The sampling of air circulating in the Challenge Room to estimate the concentration of dispersed viable microorganisms is undertaken via each of ten ports located in the exterior walls of the Challenge Room at a height of 1 m from the floor (Figure 4). A port comprises a stainless steel tube (⅜ inch internal diameter) sealed into the Challenge Room wall. The end of the tube, located in the Sampling Corridor, is fitted with a ⅜ inch stainless steel Swagelok® Quick-Connect (Crawford Fitting Company, Solon, OH) to allow attachment and ready removal of a 50 mm polycarbonate filter holder (Sartorius AG, Goeffingen, Germany). A purpose-machined stainless steel adaptor provides a leak-free link between the base of the filter holder and the plug of the Quick-Connect. On withdrawal of the plug from the Quick-Connect to access the filter holder, the port closes instantaneously, so sealing the Challenge Room. The combined filter holder, adaptor and plug of the Quick-Connect comprise a Sampling Holder. The flow of a sample of air drawn by vacuum from the Challenge Room through a filter held in the Sampling Holder is governed by a critical flow orifice located downstream of the Sample Holder. Flow is set by the diameter of the chosen orifice, diameters of 0.5, 1.0 and 1.5 mm giving flow rates of 2, 8 and 18 $dm^3$ $min^{-1}$, respectively.

**Generation of Chlorine Dioxide for Decontamination Purposes:** Following challenge studies employing microorganisms, there is a need to decontaminate the Challenge Room and contained BFS machine. Given the nature of the Challenge Room and the complex construction of the BFS machine, chlorine dioxide (CD) gas was chosen for decontamination purposes. This gas has high microbicidal, including sporicidal, activity and exhibits relatively low toxicity to humans. In addition, it is compatible with a wide range of materials including many synthetic polymers (4).

CD gas cannot be compressed and stored and thus must be generated at the point of use. For the MCF, a purpose-built CD generator was provided by Johnson & Johnson, Sterilization Science & Technology, New Brunswick, NJ. The generator produces CD gas from

the reaction of solid flake sodium chlorite and dilute chlorine gas (2% chlorine: 98% nitrogen) according to the following reaction:

$$2NaClO_2 + Cl_2 \rightarrow 2ClO_2 + 2NaCl \quad (1)$$

The generator, capable of delivering 30 $dm^3$ CD $min^{-1}$, is located within a vented cabinet in the HVAC room. The generated gas is delivered into the circulating air of the Challenge Room at the supply side of the blower. A flow of CD/air mixture, drawn through a loop on the return side of the blower, allows for real-time spectrophotometric measurement (5) of the concentration of CD present in the circulating air, which is indicative of the concentration in the air of the Challenge Room.

A separate blower and duct system provides for removal of the CD/air mixture from the Challenge Room and its dispersal to atmosphere through an exhaust that is a branch of the air circulation system. Exhausting achieves CD levels of 0.1 ppm or less before personnel are allowed to enter the decontaminated area. Additionally, during decontamination, levels of CD in the HVAC room and the Sampling Corridor surrounding the Challenge Room are measured using EIT GasPlus Series 4600 electrochemical sensors (Scott Instruments, Exton, PA) to ensure levels do not exceed 0.3 ppm, the 15 min short time exposure limit.

## Development Studies

*Chlorine Dioxide Decontamination*

**Chlorine dioxide/air mixtures in the Challenge Room:** Admission of CD gas into the air circulating within the Challenge Room and the associated HVAC system, followed by exposure to the CD/air mixture, was carried out with the room sealed, including the card exit port in the East wall, and the Changing Room doors locked. The circulating air was conditioned to between 74 and 77% RH, a level at which the microbicidal action of CD is most effective (6). In addition, light was excluded from the Challenge Room; this action prevents photolytic-induced formation of a mist from reaction of CD with water vapour (7).

Initially, studies were undertaken to examine (a) the kinetics of the build-up of CD gas concentration in the circulating air on release of the gas into the HVAC system, (b) the constancy of the final attained concentration of gas with time, and (c) the kinetics of removal of CD from the circulating air on dilution and exhausting to the atmosphere. Four final CD concentrations, 1.0, 1.5, 2.0 and 3.0 mg $dm^{-3}$, in air were studied, a typical behaviour being that of 1.0 mg $dm^{-3}$ shown in Figure 5. At t = 0 min, CD gas production was initiated by the passage of chlorine gas through the sodium chlorite cartridge that is part of the CD generator and the resultant CD gas was released into the circulating air. With increasing time, the gas concentration in the circulating air increased, achieving the desired 1.0 mg $dm^{-3}$ concentration at t = 60 min. With the gas concentration at 1.0 mg $dm^{-3}$, CD production was stopped and the air circulated in the Challenge Room for a period of 120 min during which the CD concentration remained unchanged. Following this 'holding' period, the circulating CD/air mixture was diluted and exhausted to atmosphere, thereby causing the CD concentration to fall rapidly to a recorded level of 0 mg $dm^{-3}$ at t = 330 min. The same general behaviour was seen for the other CD concentrations studied, although, as might be expected, the periods of time for build-up and removal increased with increasing concentration. This behaviour allowed investigations of the microbicidal action of CD/air mixtures to be performed with subsequent identification of an effective science-based decontamination cycle.

**Development of the decontamination cycle:** The particular CD/air mixture and the time of exposure to this mixture used for decontamination of the Challenge Room and the BFS machine (designated the decontamination cycle) were chosen from a consideration of the lethal action of various gas mixtures on spores of *Bacillus subtilis* NCIMB 8649. Spores of this organism are to be used as the test organism in certain of the future studies designed to challenge the BFS technology. They were found to have a somewhat higher resistance to inactivation by CD than spores of *Bacillus atrophaeus* ATCC 9372, the organism generally used as a biological indicator for CD gas sterilization (5).

Using purpose-made filtration equipment (Air Dispersions Ltd), nominally $10^6$ air dispersed spores were loaded onto each of a number of 50 mm water soluble gelatin filters (type SM12602 ALK, Sartorius AG). Each such filter was assembled in a Sampling Holder. A CD/air mixture at one of four concentrations (1.0, 1.5, 2.0 or 3.0 mg $dm^{-1}$) was generated in the Challenge Room and, during the 'holding period', the mixture was drawn from the room through the assem-

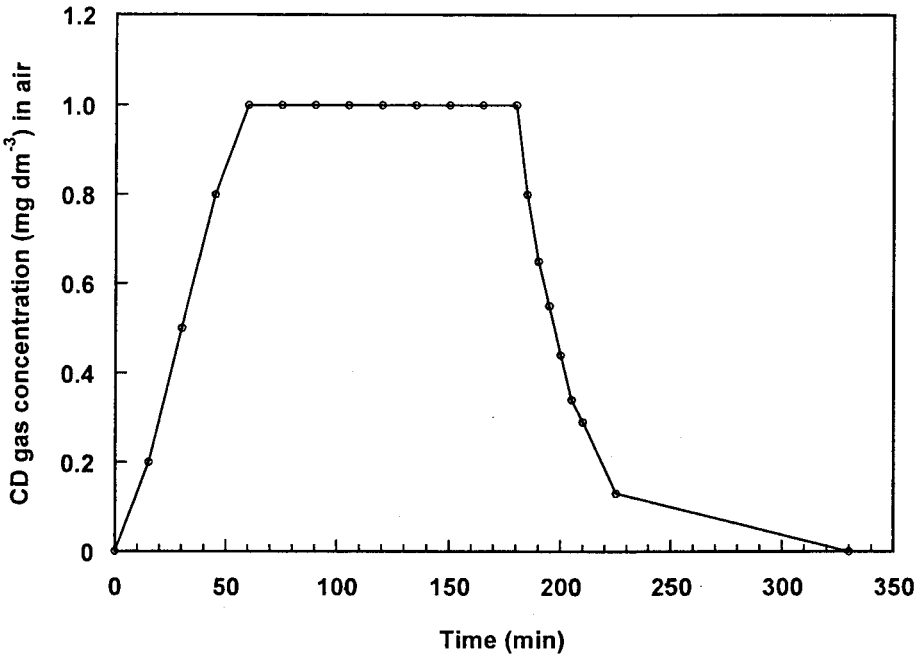


**Figure 5**

**Kinetics of attainment of a 1.0 mg $dm^{-3}$ concentration of chlorine dioxide in the Challenge Room and of CD gas exhaustion to atmosphere**

bled Sampling Holder for a pre-selected period of time at the lowest available flow rate (2 $dm^3$ $min^{-1}$) by attachment of the holder under vacuum at one of the sampling ports. After flowing through the Sampling Holder and vacuum source, the CD/air mixture was returned to the Challenge Room through an available sampling port in order not to lower CD concentration during the course of the holding period. At the end of the period, the Sampling Holder was detached from the port and air flowed through the assembly to free it of CD gas. Thus, the test spores were exposed to a particular gas concentration for a given time at room temperature falling within the range 20–23 °C.

Figure 6 is a semi-logarithmic plot of number of surviving spores against time for the four different CD concentrations. Spore inactivation is evidenced at each of the concentrations with the rate of inactivation increasing with increasing CD concentration. Times for achieving a 4 log spore reduction are roughly 30, 25, 20 and 10 min for CD concentrations of 1.0, 1.5, 2.0 and 3.0, respectively.

Given the length of time required to reach a desired CD concentration within the Challenge Room, together with that required to remove the gas from the room at the end of the holding period, the 1.0 mg $dm^{-3}$ CD level was selected as most appropriate for decontamination purposes. On the assumption that linear or near-linear inactivation kinetics hold at low probabilities of spore survival, a minimum of 15 log spore reduction was identified as effective decontamination since, in future challenges of the BFS technology, the room could be contaminated with up to $10^{12}$ spores. To meet this level of decontamination, a cycle of holding for 120 min at a CD concentration of 1.0 mg $dm^{-3}$ was considered necessary.

Confirmation of the effectiveness of this cycle was sought through performance of a further series of experiments. For each experiment, the holding period (120 min) was regarded as four equal periods of 30 min. In each 30 min, the extent of spore reduction due to a 24 min exposure to CD/air mixture was measured in duplicate, employing gelatin filters loaded with nominally $10^6$ spores of *B. subtilis* set up in the same manner as that described above. The experiment was carried out in triplicate. Table I lists the values of log spore reduction observed for the two filters exposed for 24 min during each quartile of three separate decontamination cycles. Clearly, the log spore reductions are relatively consistent within and between the various 24 min periods of exposure. The mean of each pair of values has been used to predict the log reduc-

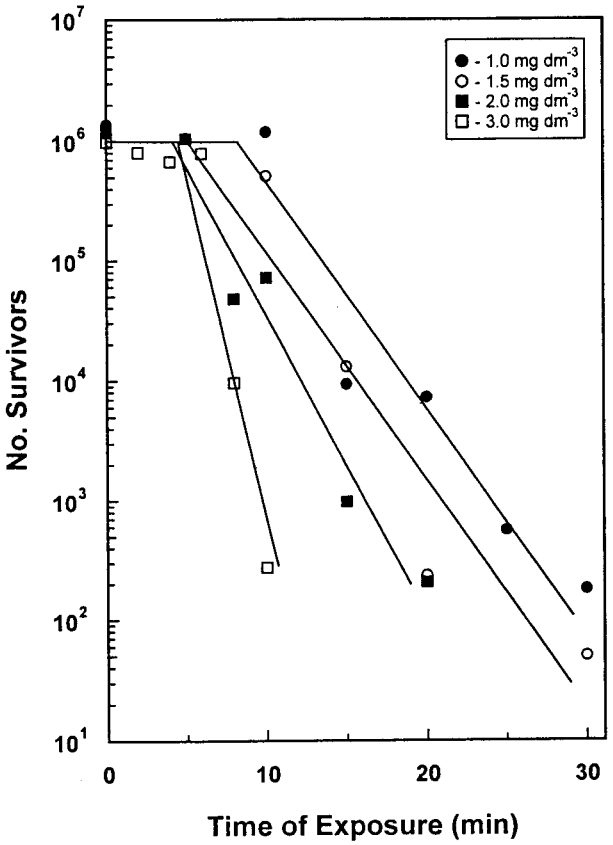


**Figure 6**

**Semi-logarithmic plot of number of surviving spores against time of exposure for four chlorine dioxide concentrations in air**

tion for the corresponding 30 min quartile period. Individual predicted values are also tabulated. Summation of these values for each cycle give overall estimates of log spore reduction for the decontamination cycle ranging from 16.65 to 21.55, levels that fall above the 15 log reduction regarded as minimum for effective decontamination.

*Air Dispersed Microorganisms*

**Suitability of the Quick-Connect:** Prior to incorporation of the Swagelok® Quick-Connect into the air sampling system, the extent of loss of microorganisms from air flowing through the connector was assessed. This laboratory assessment, performed with *B. subtilis* spores, was carried out using equipment developed for barrier testing work (8).

Discrete air dispersed spores at a known concentration were drawn from a revolving reservoir through an assembled Quick-Connect at each of three rates extending from 2 to 12 $dm^3$ $min^{-1}$. The percentages of spores collected after passage in the flowing air through the connector, each derived from five separate estimates made at a given flow rate, are given in Table II. They are indicative of little or no loss of the test spores from the flowing air. Thus, the Quick-Connect, which provides a simple and ready method for attachment of the Sampling Holder to and its removal from the Challenge Room sampling ports, will not affect significantly an estimate of the number of air dispersed microorganisms recovered from air drawn from the room.

**Production, maintenance and dispersal of *Bacillus subtilis* spores:** In generating airborne microorganisms within the Challenge Room, the nebuliser was initially charged with 300 $cm^3$ of a suspension of a given test microorganism in water at a concentration appropriate for dispersion production in the room at the desired level. Concurrently, the nebuliser recharge vessel, from which transfers would be made to maintain the suspension volume in the nebuliser, was filled with a suitable volume of the same microbial suspension. The nebuliser was operated through application of compressed air at 100 kPa and the output discharged into the HVAC ducting through which air, conditioned to between 60 and 80% RH, was flowing at 51 $m^3$ $min^{-1}$.

Monitoring of dispersed microorganisms was achieved through obtaining estimates of numbers of viable organisms present in samples of air of known volume drawn from the Challenge Room via the ten sampling ports. Four of the ports (namely, 2, 4, 7 and 9) were set up to sample air at defined locations within the interior of the room, while the remaining six drew air circulating close to the room walls. Sampling of the room interior was achieved through attaching an appropriate length of PVC tubing (9 mm internal diameter) to each of the four ports inside the room; the lengths of tubing were <5 m and were configured to avoid loss of flowing microorganisms from sampled volumes. Sampling locations are shown in Figure 7. To estimate the number of viable organisms in the sampled air, the latter was flowed through a Sampling Holder fitted with either a membrane filter (HAWGO4700, Millipore Co., Bedford, MA) or a gelatin filter (SM12602 ALK, Sartorius AG), depending on the level of microorganisms dispersed in the room. For levels up to $10^4$ $m^{-3}$, a membrane filter was used in conjunction with a nominal rate of flow of 2 or 8 $dm^3$ $min^{-1}$and enu-

TABLE I
**Observed and Predicted Log Spore Reductions Relating to Three Separate Chlorine Dioxide Decontamination Cycles**

| Quartile No. | Cycle 1 | | Cycle 2 | | Cycle 3 | |
|---|---|---|---|---|---|---|
| | Observed 24 min log Spore Red | Predicted Quartile log Spore Red | Observed 24 min log Spore Red | Predicted Quartile log Spore Red | Observed 24 min log Spore Red | Predicted Quartile log Spore Red |
| 1 | 3.50 | 5.01 | 5.57 | 7.16 | 4.66 | 5.94 |
| | 4.52 | | 5.88 | | 4.85 | |
| 2 | 2.54 | 3.74 | 3.65 | 5.34 | 4.00 | 4.75 |
| | 3.44 | | 4.89 | | 3.60 | |
| 3 | 3.19 | 3.23 | 3.54 | 4.45 | 3.30 | 5.19 |
| | 1.98 | | 3.58 | | 5.00 | |
| 4 | 3.13 | 4.67 | 4.80 | 4.60 | 2.52 | 4.84 |
| | 4.35 | | 2.55 | | 5.22 | |
| Overall predicted log spore reduction for decontamination cycle | | 16.65 | | 21.55 | | 20.72 |

meration of collected viable microorganisms carried out through a colony count obtained by direct plating of the filter on a nutrient agar medium. For levels $>10^4$ $m^{-3}$, a gelatin filter was employed at a nominal rate of flow of 2, 8 or 16 $dm^3$ $min^{-1}$ and, following recovery, it was dissolved in 10 $cm^3$ of recovery liquid. A viable count was then carried out on the resultant suspension using a standard method utilising surface spread plates.

Figure 8 shows a typical outcome of continuous provision of nebulised microbial suspension to the empty Challenge Room. In this instance, the organisms in the nebuliser were spores of *B. subtilis* (NCIMB 8649) at a concentration of $1.4 \times 10^6$ $cm^{-3}$. Nebulisation of the suspension commenced at 0 min and was continued for 600 min. Individual estimates of the number of spores were made for air samples drawn at each of the ports at 60 min and at 90 min intervals thereafter throughout the study period. It is evident from Figure 8 that, on each sampling occasion, the spore concentration estimated at the 10 distant locations, including those near the floor and ceiling of the room, fall close to one another and within a twofold range. This narrow range is indicative of air movement associated with the operation of the HVAC system acting to disperse spores homogeneously throughout the air of the Challenge Room. It is also apparent from the figure that an initial nebulisation period of at least 60 min is required to reach an effectively constant level of dispersion of around $1.3 \times 10^5$ spores $m^{-3}$ within the room and that this fixed concentration is maintained over a period of time extending from 150 to at least 600 min.

TABLE II
**Penetration of Airborne Spores Passing Through the Swagelock® Quick-Connect at Three Different Flow Rates**

| Flow Rate ($dm^3$ $min^{-1}$) | Mean Spore Penetration ± SD (%) |
|---|---|
| 2.0 | 82.0 ± 2.8 |
| 7.1 | 97.0 ± 12.3 |
| 12.0 | 95.1 ± 7.1 |

Contained dispersions of particles such as airborne spores undergo decay with time principally due to gravity (9). A form of decay known as 'stirred settling' occurs if sufficient air movement is created and, even though the dispersion is decaying under the influence of gravity, particles are uniformly distributed throughout the contained volume. Under these conditions, the rate of decay for a given mass of particle is independent of the concentration of particles, but inversely related to the height of the contained volume

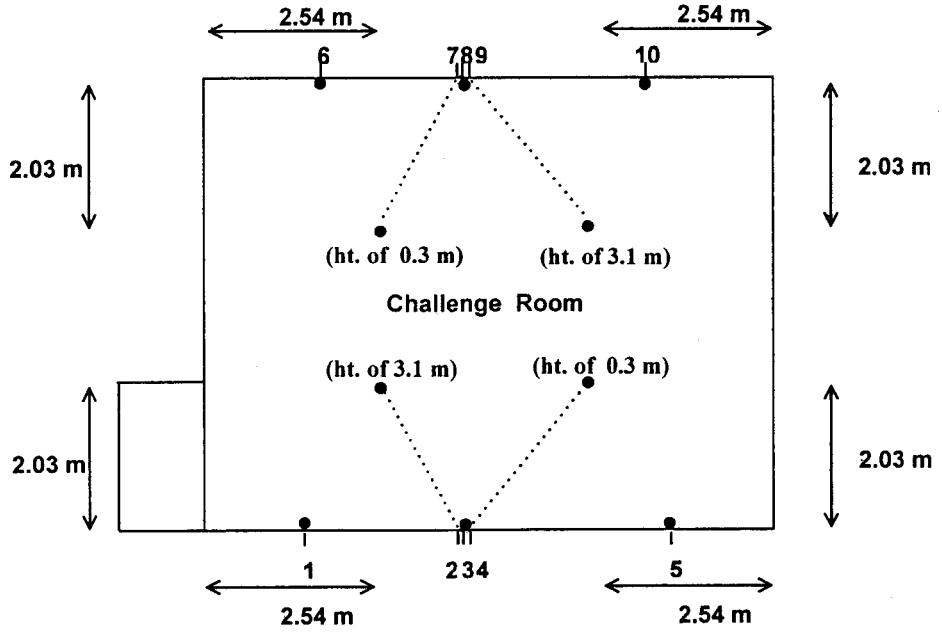


**Figure 7**

**Plan of locations for sampling air of the empty Challenge Room**

(10). Thus, the level of spore concentration seen in Figure 8 is simply a reflection of a balance between the loss of spores from the contained volume and the introduction of nebulised spores.

Figure 9 depicts the behaviour of four dispersions, each generated from continuous nebulisation of a *B. subtilis* spore suspension of different concentration (namely, $5.0 \times 10^3$, $9.1 \times 10^4$, $1.4 \times 10^6$and $8.9 \times 10^7$ spores $cm^{-3}$). Each plotted value is a mean of ten estimates of concentration obtained from air samples taken at a given time at the different sampling locations. From the figure, it is clear that nebulisation of a particular suspension gave and maintained a corre-

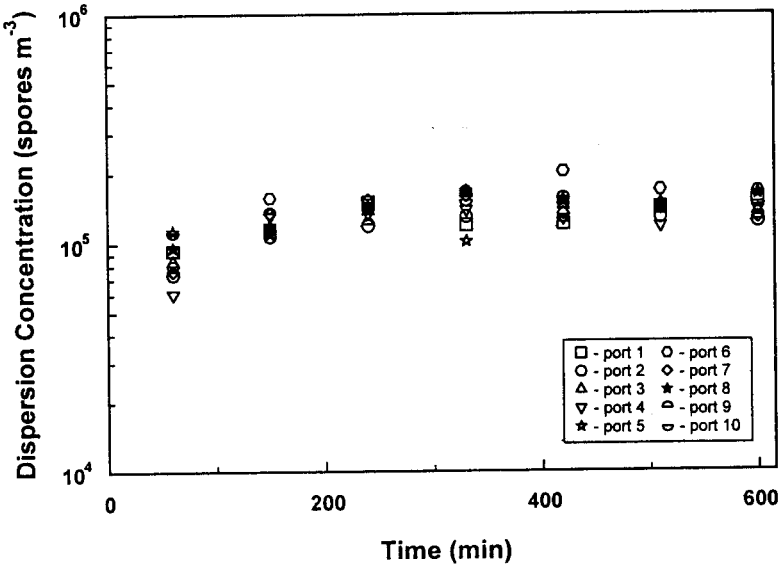


**Figure 8**

**Concentrations of spores in the air of the empty Challenge Room derived from sampling at ten distant locations during 10 h continuous nebulisation of a spore suspension of 1.4 x $10^6$ spores $cm^{-3}$**

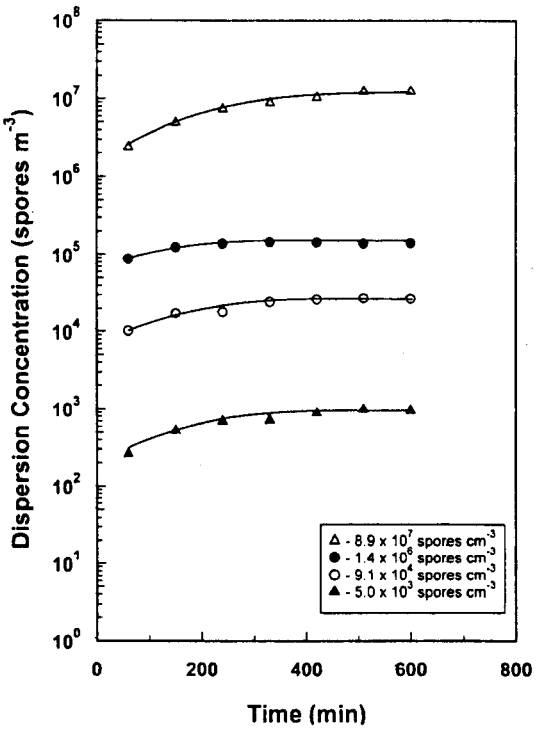


**Figure 9**

**Mean concentration of spores in the air of the empty Challenge Room derived from sampling at ten distant locations during 10 h continuous nebulisation of spore suspensions of various concentrations**

sponding given level of dispersed spores. From such studies, a direct relationship between the two variables can be developed which, in turn, allows ready production of any desired dispersion concentration within the Challenge Room through nebulisation of a microbial suspension of appropriate concentration.

**Containment of dispersed spores:** During the course of the maintenance of the above four dispersions, the effectiveness of the ACCT, operating with the conveyor belt running but without card conveyance, in preventing release of dispersed spores into the Packing and Storage Area was assessed. It is worth noting that the large opening of the ACCT (1395 $cm^2$) acts to negate pressure differences between the Challenge Room and the surrounding environment and, in so doing, precludes any driving force for air leakage from the room other than that through the ACCT. Under these circumstances, the effectiveness of the ACCT is indicative of the effective containment of dispersed microorganisms. For each of the four dispersions, a measurement of spore concentration employing filtration was made on an air sample taken at each of Port A (upstream of Stage 1) and Port B (downstream of Stage 2); see Figure 3. To ensure detection of any released spores, these samples were drawn continuously throughout the 600 min period of nebulisation. In addition, ten settle plates (Trypticase Soy Agar) were placed at well separated locations on the floor of the Sampling Corridor, Packing and Storage Area and HVAC Room.

Table IIIa lists estimates of spore concentration. As might be expected, the four concentrations in samples taken at Port A each fall close to the corresponding level occurring in the Challenge Room. On the other hand, concentration measurements at Port B show no presence of test spores for dispersion levels up to around $10^5$ spores $m^{-3}$. However, at the higher concentration of $1.1 \times 10^7$ spores $m^{-3}$ (Port A), a low level of spores corresponding to $8.7 \times 10^1$ spores $m^{-3}$ was detected, giving an estimated efficiency for the ACCT of 99.9992%. This detected level is regarded as low and acceptable. Moreover, the ten settle plates positioned for each of the four spore dispersions gen-

**TABLE III**
**Spore Concentrations Within the Challenge Room and at Ports A and B of the ACCT for Dispersions at Each of Four Different Concentrations**

| a) No BFS Machine in Challenge Room | | |
|---|---|---|
| Dispersion Concentration (spores $m^{-3}$) | | |
| Challenge Room | Port A | Port B |
| $7.3 \times 10^2$ | $7.7 \times 10^2$ | 0 |
| $2.2 \times 10^4$ | $1.9 \times 10^4$ | 0 |
| $1.3 \times 10^5$ | $1.3 \times 10^5$ | 0 |
| $8.4 \times 10^6$ | $1.1 \times 10^7$ | $8.7 \times 10^1$* |

* Corresponding to an ACCT protection efficiency of 99.9992%.

| b) Operating BFS Machine in Challenge Room | | |
|---|---|---|
| Dispersion Concentration (spores $m^{-3}$) | | |
| Challenge Room | Port A | Port B |
| $2.6 \times 10^3$ | $9.3 \times 10^2$ | 0 |
| $4.6 \times 10^3$ | $9.7 \times 10^3$ | 0 |
| $2.5 \times 10^5$ | $1.7 \times 10^5$ | 0 |
| $1.8 \times 10^6$ | $2.0 \times 10^6$ | $1.5 \times 10^1$* |

* Corresponding to an ACCT protection efficiency of 99.9993%.

erally provided 0 counts of test spores, with no plate exhibiting more than 2. The spore counts did not exceed the background counts for colony forming units (cfu) in the facility. Overall, these findings demonstrate that the ACCT provides effective containment of dispersed microorganisms within the Challenge Room with the conveyor belt running but without card conveyance. Work performed in qualification studies addresses the effectiveness of the ACCT during card conveyance.

**Other test microorganisms:** For each of four test organisms, including Gram-positive [*Micrococcus luteus* (NCIMB 8166) and *Staphylococcus epidermidis* (NCIMB 12721)] and Gram-negative [*Escherichia coli* (NCIMB 8797)] vegetative cells and a yeast cell [*Schizosacchromyces pombe* (NCYC 1364)], a suspension of a given microorganism at between $10^6$ and $10^7$ cfu $cm^{-3}$ was nebulised over a 190 min period and the resultant dispersion was monitored by air sampling at 60 min intervals at each of the 10 sampling ports. Figure 10 shows the behaviour the dispersions generated within the Challenge Room; plotted values are

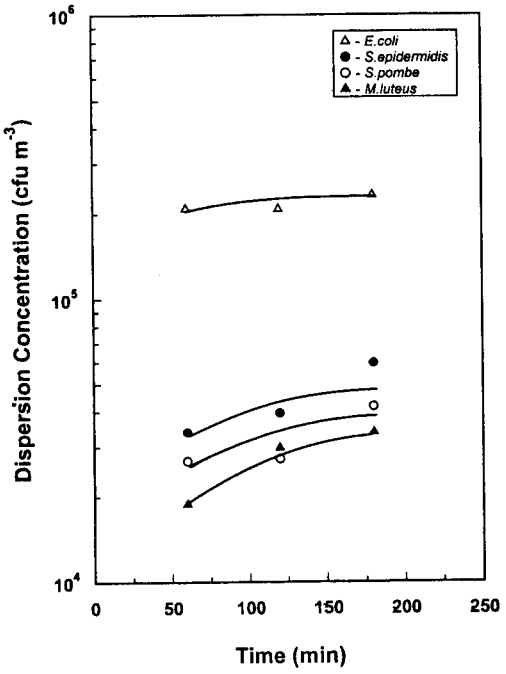


**Figure 10**

**Mean concentrations of cells of different test organisms in the air of the empty Challenge Room derived from sampling at ten distant locations during 190 min continuous nebulisation of suspensions of between $10^6$ and $10^7$ viable cells $cm^{-3}$**

**TABLE IV**
**Data on Different Test Organisms used to Generate Dispersions Within the Empty Challenge Room**

| Test Organism | Suspension Concentration (cfu $cm^{-3}$) | Mean Dispersion Concentration (cfu $m^{-3}$) |
|---|---|---|
| *M. luteus* | $5.6 \times 10^6$ | $2.8 \times 10^4$ |
| *Staph. epidermidis* | $1.6 \times 10^6$ | $4.4 \times 10^4$ |
| *E. coli* | $1.7 \times 10^7$ | $2.2 \times 10^5$ |
| *S. pombe* | $3.2 \times 10^6$ | $3.2 \times 10^4$ |
| *B. subtilis* spores* | $9.1 \times 10^4$ | $2.2 \times 10^4$ |

* Included for comparative purposes.

mean concentrations derived from estimates obtained from samples taken at the ten distant locations. It is seen that, for each test organism, it is possible to produce and maintain over time in the room a dispersion of viable cells at a relatively constant concentration.

Columns 2 and 3 of Table IV list the suspension concentrations employed in the nebuliser in this set of experiments and the corresponding overall mean concentrations of the four dispersions generated in the room, respectively. For comparative purposes, the suspension concentration of *B. subtilis* spores giving a comparable dispersion concentration to those obtained with the vegetative and yeast cell suspensions are also included in the table. Clearly, for the vegetative and yeast cells, a much higher cell concentration is required in the nebuliser than that of spores in order to achieve a given dispersion concentration. As no loss of cell viability was observed in the suspension refluxing in the nebuliser during operation, it would seem that such loss is occurring when the cells are in the airborne state. Nonetheless, it is clearly practicable to produce a wide range of microbial dispersions in the air of the Challenge Room for deployment as controlled challenges to BFS technology.

## Qualification Studies

*Decontamination of the BFS Machine*

The effectiveness of the defined CD/air mixture cycle to decontaminate a BFS machine present in the Challenge Room has been assessed through exposure of

biological indicators (BIs) located at a variety of places on and in the machine to a half decontamination cycle (60 min at 1.0 CD mg $dm^{-3}$). The BIs comprised *B.atrophaeus* ATCC 9372 spore strips (Sterris Corp., Mentor, OH), individually packaged in Tyvek pouches, at a minimum labelled population of $10^6$ spores per strip; this BI is recommended by Johnson & Johnson, Sterilization Science & Technology for monitoring the lethality of CD gas/air mixtures.

Twenty eight BIs were placed at different locations on or in the ASEP-TECH® Model 624 BFS machine (Weiler Engineering, Elgin, IL), including upper and lower machine surfaces, behind hinged machine panels and inside the machine nozzle shroud and the extruder drive exhaust filter. On completion of the half cycle, the exposed BIs were recovered from the Challenge Room and individually immersed in Trypticase Soy Broth and incubated at 30 to 35 °C for 7 days; three unexposed BIs acted as controls. All 28 exposed BIs exhibited no microbial growth and the unexposed control BIs showed positive growth. Inactivation of all of the BIs placed at the various machine locations by exposure to a half cycle of the CD/air mixture is taken as providing assurance that delivery of a full cycle will readily decontaminate all BFS machine surfaces on which test microorganisms are present.

Following the qualification study, inspection of the BFS machine, and its subsequent operation, revealed little or no impact of the CD/air mixture at the 1.0 mg $dm^{-3}$ level on the machine components and operating systems. Clearly, this is a critical finding as CD gas decontamination is to be regularly applied to BFS machinery within the Challenge Room.

*Airborne Microbial Challenges to the BFS Machine*

**Equality of spore challenges:** In order to examine the equality of challenge to the Model 624 BFS machine installed in the Challenge Room, the ends of the PVC tubing connected to the sampling ports 2, 4, 7 and 9, originally employed to sample air within the interior of the empty room, were relocated at sites within or close to what might be deemed the 'critical area' of the process. The sites were:

Port 2: Between the extruder barrel and the parison head close to where parison formation and container moulding occurs.

Port 4: Above the upper surface of the shuttling mould in which moulded containers are open to the room air.

Port 7: Within the air shroud that surrounds the filling nozzles in the 'up' position.

Port 9: Within an area, inaccessible during machine operation, in front of and on the air shroud.

The remaining six ports were used to sample air circulating close to the walls of the Challenge Room. For qualification purposes, the machine was run to fill cards, each comprising twenty four 5 $cm^3$ vials, without the air shroud surrounding the filling nozzles functioning, with card conveyance through the ACCT occurring and with one of a number of dispersions of *B. subtilis* spores in air circulating in the room at nominal concentrations ranging from $4 \times 10^1$ to $2 \times 10^5$ spores $m^{-3}$. With each dispersion concentration, air sampling was carried out at all ten sampling ports on at least three occasions separated minimally by 60 min.

Figure 11 shows the findings from four such qualification studies. The data points give values of dispersion concentration derived from air samples taken close to the process 'critical area'. On each occasion when four such separate samples were drawn, the resultant four concentrations are close to one another, indicating that active dispersal was operating to give homogeneous microbial distribution within the immediate machine environment. This finding holds irrespective of the nominal dispersion concentration. Each of the dashed lines on the figure signifies the room dispersion concentration found for the particular study. It depicts the mean of the concentrations found over the whole of the study period in air, circulating close to the room walls, sampled at the six remaining ports. Clearly, each set of data points falls on the respective dashed line, showing that, in the absence of nozzle shroud operation, microbial dispersions are uniformly distributed throughout the room air, including the surrounds of the BFS process, thereby providing equality of challenge to critical aseptic operations such as vial production, mould transfer and vial filling and sealing.

**Containment of spores:** Table IIIb lists data generated to examine the effect of card conveyance from an

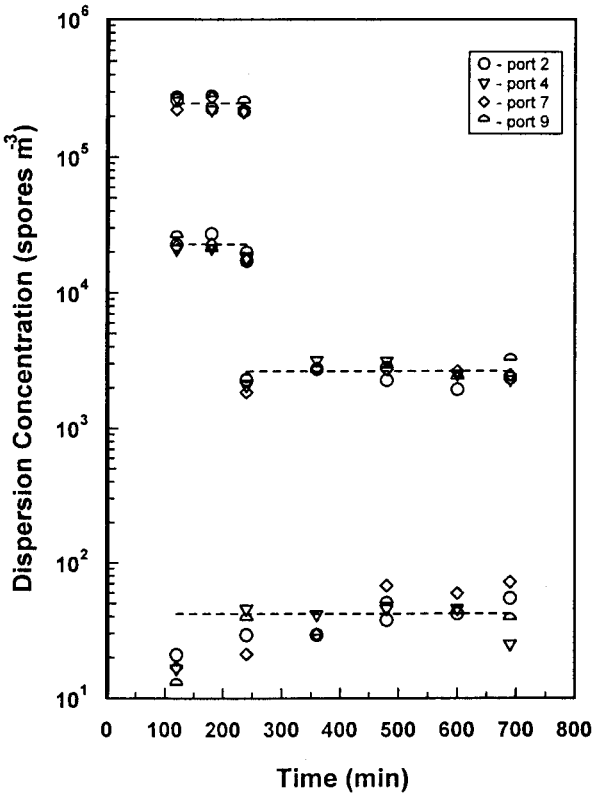


**Figure 11**

**Concentrations of spores in the air derived from sampling at four locations close to or within the 'critical area' of an operating BFS machine housed in the Challenge Room during continuous nebulisation of spore suspensions of various concentrations. Dashed lines correspond to the mean spore concentrations derived from sampling close to the walls of the Challenge Room at the remaining six ports**

operating BFS machine to the Packing and Storage Area on the effectiveness of the ACCT. Four dispersion concentrations varying over a thousand-fold range were tested. The data are essentially the same as those with spores dispersed in the empty Challenge Room with no card conveyance (Table IIIa). They demonstrate that the room containment system functions efficiently when microbial challenges are applied to a fully operational BFS machine.

**Challenges with other test microorganisms:** Each of four test organisms, other than bacterial spores, have been employed to produce dispersions in air circulating in the Challenge Room containing an operating BFS machine with the air sampling system configured as described immediately above. Table V summaries the relevant findings from these studies. For each organism, the target suspension concentration for inclusion in the nebuliser was around $10^6$ cells $cm^{-3}$, giving mean dispersion concentration in the circulating air of between $10^3$ and $10^4$ cells $m^{-3}$ (column 3). For comparison, column 4 of the table gives, for each of the test organisms, the corresponding mean of the concentrations of dispersed cells found in air samples drawn solely and intermittently from within or close to the operating machine over the study period. In all instances, there is good agreement between the two corresponding values, indicating that vegetative and yeast cells can also be distributed homogeneously throughout the Challenge Room, containing an operating BFS machine, to act as challenges to the aseptic technology.

**TABLE V**
**Homogeneity of Dispersions Generated from Different Test Organisms in the Challenge Room Housing an Operating BFS Machine**

| Test Organism | Suspension Concentration (cfu $cm^{-3}$) | Mean Dispersion Concentration ± SD (cfu $m^{-3}$) | |
|---|---|---|---|
| | | (1) | (2) |
| *M. luteus* | $1.5 \times 10^6$ | $7.5 \pm 2.6 \times 10^3$ | $6.2 \pm 3.0 \times 10^3$ |
| *Staph. epidermidis* | $2.1 \times 10^6$ | $3.2 \pm 1.3 \times 10^3$ | $2.4 \pm 0.8 \times 10^3$ |
| *E. coli* | $1.8 \times 10^6$ | $1.9 \pm 0.4 \times 10^4$ | $1.6 \pm 0.4 \times 10^4$ |
| *S. pombe* | $2.3 \times 10^6$ | $7.9 \pm 2.0 \times 10^3$ | $5.3 \pm 1.1 \times 10^3$ |

(1) estimates of dispersion concentration derived from sampling at the six sites close to the walls of the Challenge Room.

(2) estimates of dispersion concentration derived from sampling at the four sites within or close to the 'critical area' of the BFS process.

## Discussion and Future Work

BFS is regarded as an advanced method of aseptic processing (11) in that it carries out polymeric container moulding, container filling with filter sterilised liquid and container closure as a wholly integrated, automated process. In the aseptic processing mode, BFS machinery is enclosed in a controlled environment (Class 100,000) with the filling nozzles protected by an air shroud (Class 100) and is intended to function without personnel intervention. Thus, in regard to this attribute, BFS aseptic processing is ideal. However, the underlying technology is complex, being made up of numerous separate operations, any one of which could be influential in the achievement of a sterile product.

To establish that an aseptic process is under overall control, the practice is to conduct initial validation media fill exercises and, thereafter, additional exercises at regular intervals under routine processing conditions, the outcomes from which, if acceptable, are interpreted as providing evidence of process control. Demonstration of such control has been the principal basis of the claim for product sterility made widely by BFS operators.

Recently, within the aseptic processing industry, there have been attempts to identify and model the various hazardous elements of aseptic processing and, subsequently, to assign a risk rating to each of these elements through a methodical assessment of the microbiological hazard (12, 13). This allows a risk management programme to be applied to the process which, subject to appropriate modification of the high risk element(s), is expected to provide a greater assurance of sterility of the processed product. To identify and model all of the high risk elements of the complex BFS process is not possible at present, simply because basic knowledge of the process in this regard is not available. The acquisition of such knowledge is the *raison d'être* of the present work programme, part of which is the construction and qualification of the Challenge Room.

The construction and qualification of dedicated facility in which a BFS machine is located and in which known airborne microbial challenges can be uniformly generated is a significant development in respect of aseptic processing. It provides the opportunity to undertake a comprehensive programme of challenges to the BFS technology. In carrying out a challenge, the BFS machine will be set up in a particular operating configuration to fill vials with growth medium; a specific test microorganism at a known concentration will be dispersed in the air circulating in the Challenge Room and, after appropriate incubation of processed vials, a measurement made of the fraction of vials contaminated peculiar to the organism, its concentration, and machine set-up. Examination of how the contaminated fraction of vials is altered in response to systematic changes in the challenge, or in process and machine variables, will allow identification of elements of the technology that, alone or in combination, are critical in determining vial contamination. Thereafter, work will be aimed at providing an understanding of the mechanisms of air and surface borne contamination in BFS processing and how machine design can be optimised to manage risk. This substantial work programme is currently underway.

## Conclusion

A dedicated Microbiological Challenge Facility has been designed, constructed and qualified to provide controlled airborne microbial challenges to the BFS aseptic process. Uniform dispersal of selected viable microorganisms, including bacterial endospores, Gram-positive and Gram-negative vegetative cells, and yeast cells, has been achieved at fixed concentrations ranging from $10^2$ to $10^7$ cfu $m^{-3}$ air of a room housing an operating BFS machine. Monitoring the numbers of viable microorganisms in the air of the room over this wide concentration range has been carried out at various defined locations both within and outside the 'critical areas' of the process to demonstrate the equality of the microbial challenge. The facility achieves effective containment of microorganisms dispersed in the air of the room while allowing conveyance of filled vials to a location external to the room where packing and storage takes place. Conditions of treatment with a gaseous mixture of chlorine dioxide and air have been selected to ensure decontamination of the room and the contained machine exposed for extended periods to a concentration of $10^7$ viable spores $m^{-3}$ of the type to be used in future challenge studies.

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