

Performance of Blow/Fill/Seal Equipment Under Controlled Airborne Microbial Challenges

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ABSTRACT: Fundamental investigations have been carried out into the effectiveness of aseptic fill using Blow/Fill/Seal machinery. Techniques have been developed to generate and to maintain over prolonged periods, controlled microbial challenges of spores of *Bacillus subtilis* var. *niger* dispersed within the air of a containment room housing a Blow/Fill/Seal machine set-up to undertake medium fill studies. The range of spore concentrations that have been generated extends from as low as 3×10^2 to as high as 10^7 spores m^{-3} . Responses to controlled microbial challenges have defined the relationship between product contamination and microbiological quality of the machine environment. The relationship is regular and amenable to extrapolation, so that it is now practicable to specify the microbiological quality of the machine environment, together with machine operating conditions, needed to attain a Sterility Assurance Level comparable to that targeted for terminal sterilization (i.e. 10^{-6}). The impacts of mould configuration, air shower operation and location of point of fill on product contamination have also been examined.

Introduction

Previous reports have detailed initial work aimed at understanding and evaluating the impact of the microbiological quality of the environment within which a Blow/Fill/Seal machine is housed on the fraction of product contaminated (1, 2). Two principal findings emerged from this work:

- There is a definable direct relationship between the fraction of product contaminated and the level of micro-organisms in the air surrounding the machine, and
- a protective air shower unit assembled around the filling mandrels reduces substantially the extent of product contamination.

With these early experiments, practical considerations dictated that the lower limit of the airborne spore challenge around the machine was 10^4 organisms m^{-3} . Given this restriction, definition of the relationship between the fraction of product contaminated and the concentration of the microbial challenge was realised over a significant, albeit somewhat limited, range of challenge concentration (~ 500 fold).

The present work was designed to confirm the nature of the relationship between the fraction of product contaminated and the challenge level and to extend the relationship to challenge concentrations in the region of 10^2 spores m^{-3} . Additionally, it aimed at establishing whether or not the fraction of product contaminated

under controlled challenge conditions is affected by a) different mould configurations, b) the mode of operation of the air shower, and c) the location of the point of fill in the filling zone.

Materials and Methods

Study Set-up

The test methods, the basic test design, the test organism and the Blow/Fill/Seal machine containment room were identical to those described before (1, 2).

The containment room was sited at Weiler Engineering, Elk Grove, Arlington Heights, Chicago, a location where no sterile pharmaceutical production is carried out. A series of challenge tests, spread over 9 successive days, was conducted on Blow/Fill/Seal machine ALP 624-017 tooled to the same specification and mould configuration (designated A), as those of ALP 624-015, the machine used in our previous work (1). Configuration A provided 24 moulded ampoules each with a 2 cm^3 fill volume; during filling, each ampoule presented a circular opening of 4.03 mm diam. to the filling mandrel. The operation of ALP 624-017 was identical to that of ALP 624-015, namely production of 24 ampoules every 12s (1 cycle) to give an overall production of 120 ampoules min^{-1} . A second test series occurred after an interval of 3 months and it encompassed challenge tests, done over 5 successive days on a different but basically identical machine (ALP 624-029), tooled to a mould configuration that provided 12 ampoules each of 10 cm^3 fill volume (configuration B); ampoules produced by mould configuration B presented an opening of 5.08 mm diam. Machine operation was fixed at 1 cycle per 13s giving a production rate of around 55 ampoules min^{-1} .

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The same air shower unit was employed on both machines to provide local protection of the filling mandrels in challenge studies when this was desired. The basic design of the air shower unit was as described previously (1). Before using a particular machine, the HEPA filter within the air shower unit was DOP tested (IES-12P-CC-002-86) and rated at 99.99% efficient. By controlling the speed of the fan located in the unit, the velocity of filtered air emerging from the air shower outlet, a slot running the length of the base of the air shower unit, was varied between 1.6 and 3.7 m s⁻¹; at a given fan speed, the velocity of the emerging air was found to be constant over the entire length of the slot.

For each challenge test, the Blow/Fill/Seal machine was set up according to the machine manufacturer's protocol for 'medium-fill validation.' The fill medium was heat sterilized Tryptone Soya Broth, tested to meet minimum USP fertility level. The medium was delivered to the point of fill after passing through two in-line filters (nominal pore size 0.2 µm) that were tested for integrity at the end of each day's experimentation.

Microbial Challenges

Air-dispersed spores of *Bacillus subtilis* var. *niger* (NCIMB 8056) comprised the microbial challenge. Aerosolisation of an aqueous spore suspension at a predetermined concentration gave spores dispersed throughout the air of the machine containment room at a given challenge concentration over the range 3 × 10² to 3 × 10⁶ viable spores m⁻³. The test duration, during which the challenge spore concentration was maintained at a given nominal level (see 3. under *Test Design* below), varied according to challenge concentration, the lower the concentration the longer the duration (a longer test duration being required at low spore challenge concentration to allow detection of the frequency of ampoule contamination). In practice, test durations ranged from 1 to 10 h. The concentration of spores in the air of the containment room was monitored intermittently during each challenge test by collecting the spores present in sample volumes of air, drawn isokinetically, from the room via three sampling ports (1).

Test Design

Irrespective of test conditions, the following activities were carried out throughout the duration of a challenge test:

1. Continuous aerosolisation of appropriate spore suspension.
2. Continuous operation of the Blow/Fill/Seal machine employing medium fill.
3. Periodic sampling of the containment room air. A minimum of four sampling operations, made up of replicate samplings at each of the three access ports, were carried out for each challenge test. For a given test, the nominal challenge concentration is the mean value of the spore concentrations derived from individual estimates made at the different sampling locations during the overall test period.

To allow measurement of the fraction of product contaminated, expressed in terms of the ratio of number of contaminated ampoules to total number of ampoules produced, each individual ampoule was identified relative to its time of production and filling location. Immediately after production, all ampoules were incubated at 30–35°C for 14 days so that contamination of ampoules could be assessed by appearance of visible growth.

Results

The Relationship between Fraction of Product Contaminated and Level of Spore Challenge

As part of the present study, challenge tests have been carried out on an ALP 624 Blow/Fill/Seal machine of the same type as that used on the previous occasion, set up as before (configuration A) and operated under exactly the same conditions (1). The difference in the nature of the tests performed on the two occasions employing configuration A lies solely in the different levels of spore challenge employed, which, in turn, influenced the numbers of ampoules filled when levels of challenge were relatively low. This is evident from a consideration of Table 1. For any given challenge test, a minimum target of 3 contaminated ampoules was set. This minimum level gives predicted upper and lower 95% confidence limits, about the fraction of ampoules contaminated, that cover a 5 fold range. In practice, the numbers of ampoules filled ensured that the minimum of 3 was always exceeded, which, in turn, provided a range for the 95% confidence limits that fell below the 5 fold range.

In conducting a series of tests on a given occasion, the series was started with the test employing the lowest spore concentration and then continued with tests using successively increasing concentrations; this design avoids potential contamination of ampoules by spores present in the environment at high numbers from a previous test. Moreover, each successive test performed at a given spore challenge level was preceded by filling a minimum of 7200 ampoules (equivalent to 1 h machine operation)

TABLE I
Characteristics and Results of Challenge Tests Performed on Two Separate Occasions with the Same Type of Blow/Fill/Seal Machine Tooled to a Given Mould Configuration (A) and Operated Identically

	Challenge Concentration (Spores/-m ³)	Number Ampoules Filled	Number Ampoules Contaminated	Fraction Ampoules Contaminated
Occasion 1 (Previous)	4.1 × 10 ⁴	9528	129	1.35 × 10 ⁻²
	1.4 × 10 ⁶	7800	2090	2.68 × 10 ⁻¹
	1.7 × 10 ⁷	9384	9242	9.85 × 10 ⁻¹
Occasion 2 (Present)	3.34 × 10 ²	71688	7	9.76 × 10 ⁻⁵
	1.23 × 10 ³	15336	4	2.61 × 10 ⁻⁴
	6.08 × 10 ⁴	9072	107	1.18 × 10 ⁻²
	2.02 × 10 ⁶	4248	1855	4.37 × 10 ⁻¹

without aerosolisation of spore suspension and in no such instance was a contaminated ampoule found.

Figure 1 is a plot, on logarithmic scales, of the fraction of contaminated ampoules against spore challenge concentration for the machine (configuration A) operating without the fan in the air shower unit functioning. The three open points represent previously reported data (1) and the four closed points represent the data generated in the present study. It is clear from the figure that both sets of datum points fall around the same curve (the dashed line is the extrapolation of the derived curve beyond the point where measurements were made). The new datum points confirm the previously described relationship between fraction of product contaminated and spore challenge concentration; the previous and new points together show that the linear portion of the curve defining the direct relationship is experimentally demonstrable over a 7000 fold change in challenge concentration.

Mould Configuration

Data, of the type shown in Figure 1, were generated for an 624 Blow/Fill/Seal machine tooled to mould configuration B and operated without the fan in the air shower unit functioning. Individual datum points are given in Figure 2. The dashed line is the curve, taken from Figure 1, defining the relationship between the fraction of product contaminated and spore challenge concentration for mould configuration A. The five datum points fall close to and around the dashed line, indicating that the responses to changing spore challenge concentration for mould configurations A and B are indistinguishable.

Operation of the Air Shower Unit

The Blow/Fill/Seal machines were run with mould configurations A and B under the full protection of the air shower unit, i.e. with the fan speed set at maximum. The velocity of air emerging from the air shower outlet

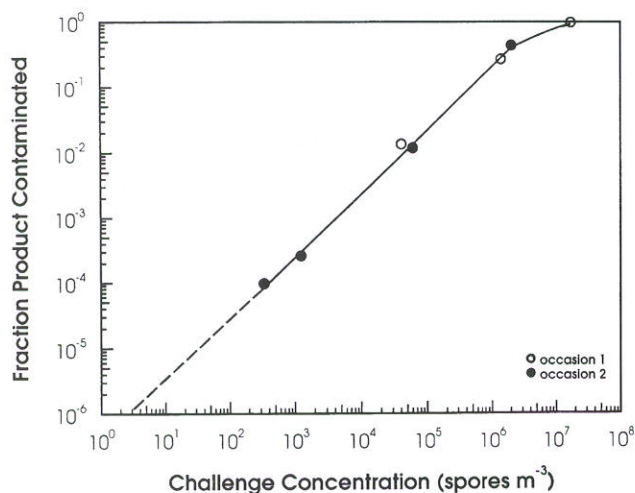


Figure 1—Fraction of ampoules contaminated as a function of spore challenge concentration for an ALP 624 Blow/Fill/Seal machine operating under a given set of conditions comprising mould configuration A, a cycle time of 12 s, and without the fan located in the air shower unit functioning.

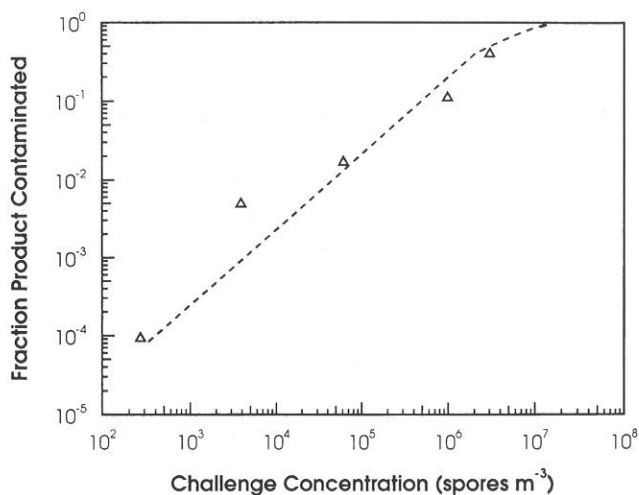


Figure 2—Fraction of ampoules contaminated as a function of spore challenge concentration for an ALP 624 Blow/Fill/Seal machine operating under a second set of conditions, namely mould configuration B, a cycle time of 13 s, and without the fan located in the air shower unit functioning. The dashed line is a representation of the solid curve given in Figure 1.

for both mould configurations was the same, measured at 3.7 m s⁻¹. The closed circles in Figure 3 represent the data for mould configuration A and the closed triangles those for configuration B. The dashed line is the curve describing the common behaviour for both mould configurations with the air shower fan not operating (taken from Fig. 2). The data indicate that, for each mould configuration, there is a distinct curve describing the relationship between fraction of product contaminated and spore challenge concentration. Furthermore, the two curves derived with the fan operating, and that describing the behaviour with the fan not functioning, appear to be parallel. However, the curves generated with the fan operating are shifted downwards from that seen with the fan off, the magnitude of this shift being

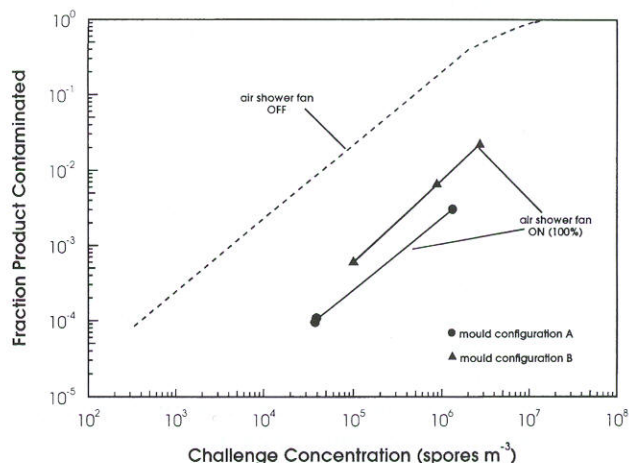


Figure 3—Fraction of ampoules contaminated as a function of spore challenge concentration for ALP 624 Blow/Fill/Seal machines operating with two different mould configurations and with the fan located in the air shower unit functioning maximally. The dashed line, describing the relationship for both mould configurations without the fan functioning (taken from Fig. 2), is included for comparative purposes.

around 30 and 90 fold for mould configurations B and A respectively.

Figure 4 shows data generated to examine the influence of the speed of the air shower fan, and hence the velocity of flow of air, on the degree of protection afforded by the air shower unit; these datum points were generated for mould configuration A at a given nominal challenge concentration (5×10^4 spores m^{-3}). The figure depicts a plot, on semi-logarithmic scales, of the fraction of product contaminated against the velocity of air measured at the outlet of the air shower unit. At an air velocity of $0 m s^{-1}$, the fraction of product contaminated is around 1.2×10^{-2} and, with increasing air flow, this value is seen to decrease progressively to a level of around 1.1×10^{-4} at a velocity of $3.7 m s^{-1}$ (the highest attainable at maximum fan speed).

Location of Point of Fill

Figure 5 shows data generated to examine whether or not the location of the point of fill along the mould block is influential in respect of product contaminated. The data were generated by processing ampoules at each of twelve in-line locations of mould configuration B while challenged at a concentration of 3×10^6 spores m^{-3} with the air shower fan off and with it operating maximally. The histogram in Figure 5 records, on a logarithmic scale, the fractions of contaminated ampoules at each of the twelve separate filling locations (designated 1–12, front to back). It is noted that, for the two modes of fan operation, contamination of ampoules occurred at all twelve points of fill and that at each fill location the operation of the air shower unit caused a significant reduction in the fraction of ampoules contaminated (on

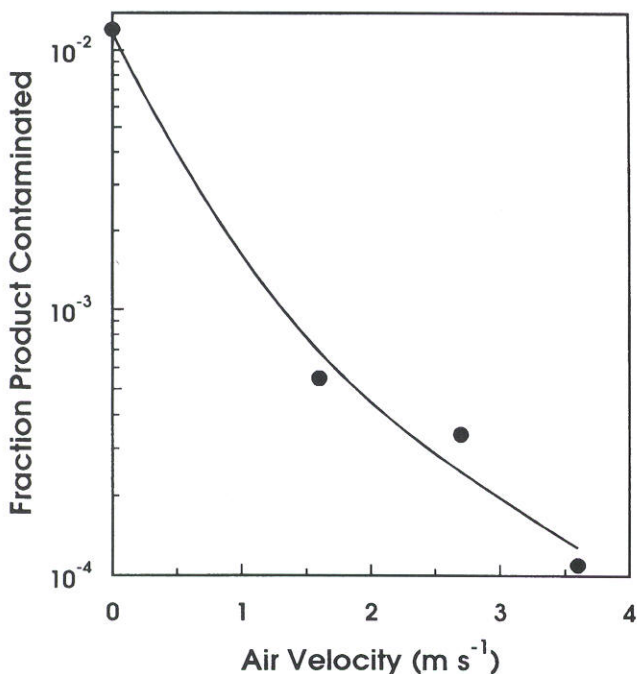


Figure 4—Fraction of ampoules contaminated as a function of the velocity of air at the outlet of the air shower unit for a Blow/Fill/Seal machine operating under a given set of conditions and challenged with a fixed spore concentration of 5×10^4 spores m^{-3} .

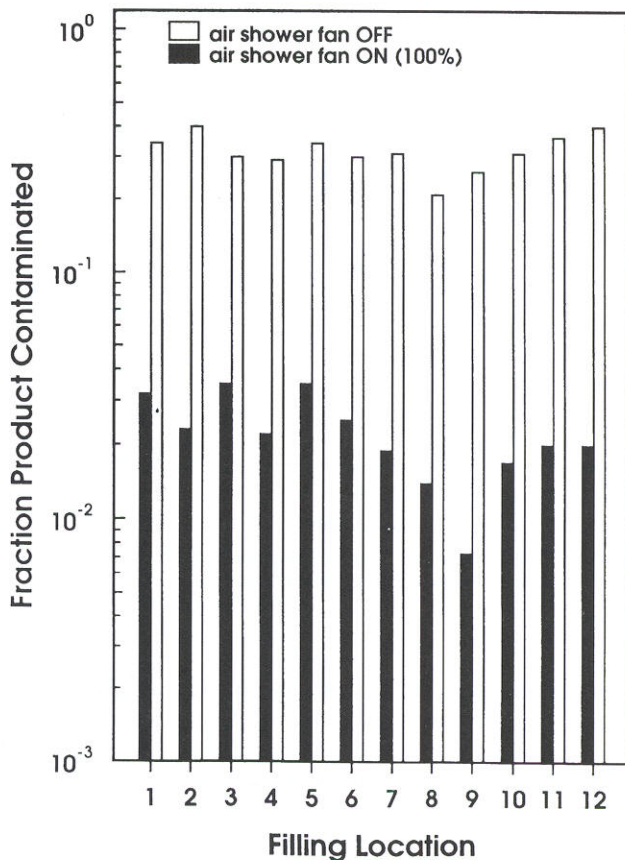


Figure 5—Fractions of ampoules contaminated at each of twelve filling locations of an ALP 624 Blow/Fill/Seal machine, operating with the air shower fan both off and one at maximum and challenged with a fixed spore concentration of 3×10^6 spores m^{-3} .

average 16 fold). However, with the fan off, the twelve fractions fall within a 2 fold range (0.21 to 0.40) whereas, with air shower on, they fall within a 5 fold range (0.0073 to 0.035). For both modes of air shower operating the differences seen between filling locations are greater than those which can be attributed to chance ($p = 0.05$). Thus, it appears that, at a given spore challenge concentration, contamination rates differ from filling location to filling location.

With the fan operating at maximum and with the spore challenge set at nominally $3 \times 10^6 m^{-3}$, data similar to that shown in Figure 5 were generated on two further occasions. Figure 6 shows fractions of ampoules contaminated at the twelve filling locations on the three separate occasions; the dashed line depicts mean levels of product contaminated at the different locations. At any given filling location, fractions of product contaminated are seen to fall within a 2 fold range whereas mean levels of contamination at the different locations vary up to 6 fold (the highest and lowest levels of contamination occurring at locations 5 and 9 respectively). Analysis of data generated on the three occasions confirms differences between fractions of ampoules contaminated at different filling locations to be significantly greater than those observed within locations ($p = 0.05$). Given this, the dashed line provides a profile of contamination characteristic for the twelve filling locations comprising

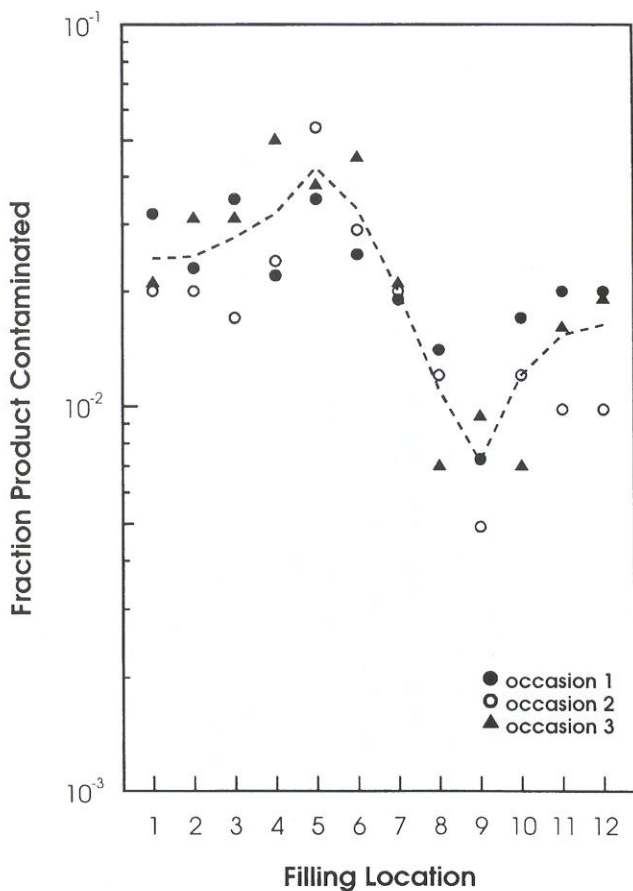


Figure 6—Fractions of ampoules contaminated at each of twelve filling locations of an ALP 624 Blow/Fill/Seal machine operating with the air shower fan functioning maximally and challenged, on three separate occasions, with a fixed spore concentration of 3×10^6 spores m^{-3} .

configuration B under a given set of operating conditions.

Discussion

Results of challenging operating Blow/Fill/Seal machines with air-dispersed spores have demonstrated, as previously reported (1), unequivocal evidence of the profound impact of the level of airborne micro-organisms in the machine environment on the fraction of product contaminated. In general, for a given mould configuration operating under fixed machine conditions, there is a strong definable relationship between the fraction of product contaminated and the level of airborne micro-organisms.

Previous work (1) defined the form of the relationship between product contamination and spore challenge concentration for mould configuration A over a limited range of spore challenge concentration (~ 500 fold range). Present work has experimentally demonstrated that, with the fan located in the air shower unit off, the previously observed direct relationship is extendable to lower levels of spore challenge concentration; the lower limit of spore challenge concentration has been set at around 3×10^2 spores m^{-3} in present work as opposed to around 4×10^4 spores m^{-3} in earlier work. Overall, the relationship for mould configuration A has been defined

over a 50,000 fold range of spore challenge concentration. Moreover, the relationship is regular and amenable to extrapolation and provides a means for predicting operating conditions under which the frequency of product contamination is low and acceptable (see dashed line on Figure 1). For example, for mould configuration A, a level of 3×10^0 spores m^{-3} in the machine environment is predicted to provide a frequency of product contamination of 1 in 10^6 . In addition, the constancy of the behaviour for mould configuration A between the two studies provides strong evidence that, under controlled conditions, Blow/Fill/Seal machine performance in respect of product contamination is highly consistent and reproducible.

Comparable data generated for mould configuration B revealed a strikingly similar behaviour to those observed for mould configuration A. In practice, the curve describing the relationship between product contamination and spore challenge concentration derived from configuration A is also a good description of that for configuration B (see Fig. 2). On the face of it, this finding is somewhat surprising given the distinctive physical differences between the product derived from the two mould configurations (24×2 cm³ ampoules for A compared with 12×10 cm³ ampoules for B). However, the apparent independence of product contamination on mould configuration might simply reflect the similarity in dimensions of ampoule openings during the filling cycle (4.03 mm for A as opposed to 5.08 mm for B). Alternatively, the common behaviour for the two configurations might be a reflection of the summation of the impacts of a number of crucial variables (such as cycle time, fill volume and speed and ampoule geometry) on product contamination. Clearly, further work is necessary to understand fully the influence of mould configuration on contamination rate.

The operation of an air shower unit around the filling mandrels was previously reported to reduce the frequency of product contamination; for mould configuration A, maximal operation of the air shower fan to give an air velocity of 4.0 m s^{-1} resulted in a 10 fold reduction in product contamination (1). In the present study, local protection of the filling mandrels is again seen to reduce the level of product contamination. However, for mould configuration A, maximal operation of the air shower unit peculiar to this study (face velocity of 3.7 m s^{-1}) brought about a 90 fold reduction in the level of product contamination. The difference in the levels of protection afforded by the different air shower units (10 and 90 fold for the previous and present studies respectively) suggests that, for a given mould configuration, the design and assembly of the air shower unit is critical to the effectiveness of the unit in providing local protection. Furthermore, maximal operation of the fan located in the air shower unit with mould configuration B brought about a 30 fold reduction in level of product contamination as opposed to 90 fold reduction for the same unit operated identically with mould configuration A. This provides evidence that mould configuration is pertinent

to the effectiveness of local protection afforded by a given air shower unit surrounding the filling mandrels.

The rate of flow of air emerging from the slot of the air shower has also been identified to be a critical determinant of the effectiveness of local protection. At a given spore challenge concentration, a strong inverse relationship between fraction of product contaminated and air velocity was observed. The form of the inverse relationship suggests that further increases in air flow, beyond those achievable to date, would enhance local protection appreciably. Furthermore, the nature of the relationship is in keeping with local protection, provided by filtered air emerging from the air shower, being achieved in part through establishing a 'compartment of clean air' within which critical Blow/Fill/Seal operations are conducted. Given the existence of such a compartment during local protection, there is a strong argument for monitoring levels of airborne micro-organisms within this location as opposed to the general machine environment. Particularly, as in time, it may be possible to effect local protection so that the level of product contamination is independent of general machine environment.

Data generated to examine the influence of the location of the filling operation have established that, for a machine operating with the air shower either off or on at maximum, ampoule contamination occurred at all filling locations. Differences between contamination rates at the different filling locations were small (up to a maximum of 5 fold), but nonetheless significant. Furthermore, a distinctive profile of product contamination at different filling locations was evidenced for configuration B with air shower on. Clearly, further work is necessary to explain fully this behaviour.

The series of investigations reported above provides a major step forward towards rationalising the application of Blow/Fill/Seal technology in aseptic processing. In summary they reveal that:

- a) the microbiological quality of the Blow/Fill/Seal machine environment is relevant to the fraction of product contaminated.
- b) the relationship between fraction of product contaminated and the level of airborne micro-organisms is regular and amenable to extrapolation and this allows prediction of operating conditions un-

der which a low acceptable frequency of contamination is attained.

- c) under controlled conditions, Blow/Fill/Seal machine performance is highly consistent and reproducible.
- d) a protective shower of air around the filling mandrels reduces the frequency of product contamination; the effectiveness of local protection is dependent upon air shower design, mode of operation and machine configuration.
- e) differences in contamination rates at different filling locations are small but nonetheless significant ($p = 0.05$), giving rise to a characteristic profile of contamination for a given mould configuration.

The work also serves to demonstrate that controlled airborne microbiological challenges provide an effective tool to allow rationalisation of machine design and conditions of machine operation. Moreover, responses to controlled microbial challenges provide the opportunity to define operating and environmental conditions under which Blow/Fill/Seal machinery can meet a Sterility Assurance Level comparable to that targeted for terminal sterilization (i.e. 10^{-6}).

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