Requalification of a cleanroom garment system

taking into account the typical signs of ageing

dastex REINRAUMZUBEHÖR

Based on a question during an audit by the authorities at a pharmaceutical company regarding qualification data for cleanroom garments of cleanroom zone A/B, this cleanroom garment was requalified* as part of a joint project. The people involved report here on the most important results and their interpretation.

* At the time of the audit by the authorities, the cleanroom garments had already been in use in cleanroom zone A/B for several years. The correct functionality of the cleanroom garments was indirectly verified by means of microbiological personnel and environmental controls, but an initial qualification of the cleanroom garments according to current requirements (or: interpretation) was not available. Due to the already established use of the cleanroom garments, this study is referred to as a requalification of the cleanroom garments.

1. Introduction

How often can a cleanroom garment be used? A question that is asked more and more frequently in audits and, in many cases, cannot necessarily be answered immediately. The background to this question is the easily comprehensible finding that the performance of a washable cleanroom garment decreases over time with the increasing number of decontamination (=washing & drying) with subsequent sterilisation cycles, as well as wear cycles, due to age-related effects. This applies all the more if the clothing is additionally sterilised. The contamination control strategy required in the draft (2020) of the new Annex 1 (GMP Guideline) also demands that all parameters that could result in contamination of the product are designed in such a way that contamination of the product can be ruled out. Of course, the production personnel pose a high microbiological and particulate contamination risk. One way of reducing the contamination risk posed by personnel is to use cleanroom garments as the only filters between people and the product. Consequently, cleanroom garments are of particular importance. The selected cleanroom garment as an essential component of the contamination control strategy must therefore be checked/qualified, evaluated and, if necessary, optimised.

In 2018/2019, an international pharmaceutical producer as a user of cleanroom garment, the cleanroom garment manufacturer Dastex with its site in Muggensturm (Germany) and the textile service provider Initial with its site in Reutlingen (Germany) took a closer look at the question of how long cleanroom garments (for sterile production in cleanliness zones A/B at a Swiss site of the pharmaceutical producer) can be used. As part of a joint project, the aim was to study how cleanroom garments behave over the maximum defined number of wearing, decontamination and sterilisation cycles. In more precise terms: whether they change qualitatively over the period of use.

The aim was to obtain reproducible values for the following parameters under conditions that are as close to practice as possible:

- Airborne particle emission from people wearing cleanroom garment of different phases of ageing
- Viable contamination at defined sampling points on the outside of persons in a cleanroom garment (textile surface)

The cleanroom garment system defined at the pharmaceutical manufacturer for sterile production in cleanliness zones A/B was introduced there several years ago and has become well established since then. The results from microbiological personnel monitoring (surface checks on the outside of the cleanroom garments at the end of the stay in cleanliness zones A/B) showed only a small number of warning and action limit violations since the introduction of this cleanroom garment system and was therefore interpreted as a reliable cleanroom garment system. However, the qualification of this cleanroom garment system was missing until now.

The relevant cleanroom garment system of the pharmaceutical manufacturer involved in this project is specified as follows:

- 2-piece cleanroom compatible undergarment, consisting of a long-sleeved top and trousers, plus
- 3-piece garment consisting of a hood, coverall and overboots.

The undergarments are made of a cleanroom compatible material woven from 100% polyester microfibre filaments. The garments are made of a material consisting of 98% polyester and 2% carbon, which has proven itself in cleanroom applications for many years.

The supplementary components of the cleanroom garment system include cleanroom suitable socks, defined cleanroom shoes, cleanroom goggles, non-woven under cap and disposable face mask, as well as two pairs of sterile gloves, each with long cuffs (length: 12 inches) that can be pulled over the sleeves of the coveralls.

The maximum number of wearing, decontamination and sterilisation cycles for garments is defined as 60 and for over boots as 40 (note: due to mechanical stresses during the decontamination process caused by the boot soles, wear-out is higher for overboots than for garments).

The clothing manufacturer Dastex has well-founded technical data that also includes signs of ageing (caused by recurring reprocessing. In addition, Dastex published a study in 2017 on the signs of ageing after 60 decontamination and sterilisation cycles – but without wearing the cleanroom garments in between (Alterungserscheinung steriler Reinraumbekleidung, ReinRaumTechnik 1/2017, Wiley). The results of this study did show a decrease in filtration efficiency; however, this was by no means to an extent that could result in a negative microbial or particulate impact on the environment.

2. Material and methods

For a comparative study – cleanroom garment in new condition versus cleanroom garment with a maximum number of reprocessing (washing/sterilising) with an intervening use (wearing) – the use of the Body-Box method with constant ambient conditions is appro-

Body-Box

Measuring system BioTrak®

Contact plate test

priate. The basics of the Body-Box method are described in an American recommendation practice (IEST RP-CC003.4). The Body-Box system is described in detail in the publication „Body-Box-Test, eine Testmethode auf dem Prüfstand" (ReinRaumTechnik 2/2004, GIT-Verlag). The environmental conditions in the Body-Box used correspond to those of a cleanliness zone ISO 4 (ISO 14644-1).

Within the framework of this study, which was carried out in the Body-Box of the company Dastex, the following activities of a person dressed in the cleanroom garment described (in different states of age) were accompanied by microbiological measurements and particle measurements. The measurements were intended to test the permeability or retention capacity of the cleanroom garment with regard to particulate and microbial contamination.

A person trained in the correct donning of cleanroom garment performed the following activities in the Body-Box:

- Entering the Body-Box
- Acclimatisation time in the Body-Box without movements
- Start of the defined movement programme (alternating walking movements and standing still). These activities were to simulate wearing the cleanroom garment.

Phase	Duration
Acclimatisation time (Standing)	**1 – 10 min**
Standing	5 min
Walking	5 min
Standing	10 min
Walking	10 min
Standing	5 min
Walking	5 min

Table 1: Movement programme: activities performed in the Body-Box

- During the movement programme:
  - Measurement of particle release (airborne; Optical Particle Counter; 28.3 L sample volume per minute; using BioTrak® and
  - Measurement of the release of viable contaminants (airborne; laser-induced fluorescence; 28.3 L sample volume per minute; using BioTrak®)
- Before leaving the Body-Box, the bacterial count on the outside of the cleanroom garment is checked by means of contact plate tests at 20 measuring points distributed over the hood, coverall and overboots:
  - 1x forehead
  - 1x cheek
  - 2x abdomen
  - 8x arms (2x upper arm and 2x lower arm each)
  - 4x legs (1x front thigh and 1x back thigh each)
  - 2x boots (1x per boot)
  - 1x bottom
  - 1x chest

The BioTrack® real-time viable particle counter enables the detection of the total particle count of airborne particles as well as a differentiation into airborne particles and airborne viable particles based on biofluorescence. The microbiological analyses were incubated at 30 – 35°C for 5 – 7 days and analysed with regard to the total bacterial count. In addition, the residual contamination levels of the reprocessed cleanroom garments were determined prior to sterilisation with the help of the suction method in accordance with ASTM-F51. In this method, air is sucked through the cleanroom fabric by means of a particle measuring device; existing particles are thereby loosened from the fabric and measured. In total, the following measuring points were defined: coverall (chest, upper arm, abdomen, front thigh, back thigh); hood (forehead, cheek (mouth area); boots (at the top of the shaft).

Three age conditions of the cleanroom garment were compared:

1. New condition ➜ cleanroom garment was professionally washed and sterilised three times (to remove potential residual substances from the fabric).
2. Final condition ➜ cleanroom garment that has reached the specified maximum number of wear cycles (60/40 cycles with a maximum of 3.5 hours wear time) incl. washing and sterilisation cycles (123 °C for 30 min.)
3. Safety buffer ➜ cleanroom garment that has reached the specified maximum number of wearing cycles (60/40) incl. decontamination and sterilisation cycles (123 °C for 30 min.) has been decontaminated and sterilised a further 20 times, but without wearing the cleanroom garments in between. These additional decontamination and sterilisation cycles of the cleanroom garment was intended to represent a worst-case scenario, on the basis of which the performance of the garment concept was also to be tested beyond the defined final cycle number.

For each ageing stage, ten sets of garments were examined both for the analysis in the Body-Box and for the determination of the residual contamination levels, in order to obtain a sufficient data basis for a statistical evaluation of the results.

3. Results

3.1 Residual contamination values

The determination of the residual contamination levels by the laundry showed that the levels – even with increasing age of the garments – remained approximately constant and were always well below the typical limits (less than 1,000 particles ≥ 5 µm/ft²) according to ASTM F51.

3.2 Microbiological results

The analysis of the microbiological load on the outside of the cleanroom garments was

carried out by the pharmaceutical manufacturer. For the statistical analysis, the results of the individual test points from the outside of the cleanroom garment were not considered separately, but were combined into one group. Figure 1 shows the individual data with the box plot of the clothing sets with 0 and 60 resp. 40 decontamination, sterilisation and wearing cycles with 0 and 60 resp. 40 decontamination, sterilisation and wearing cycles.

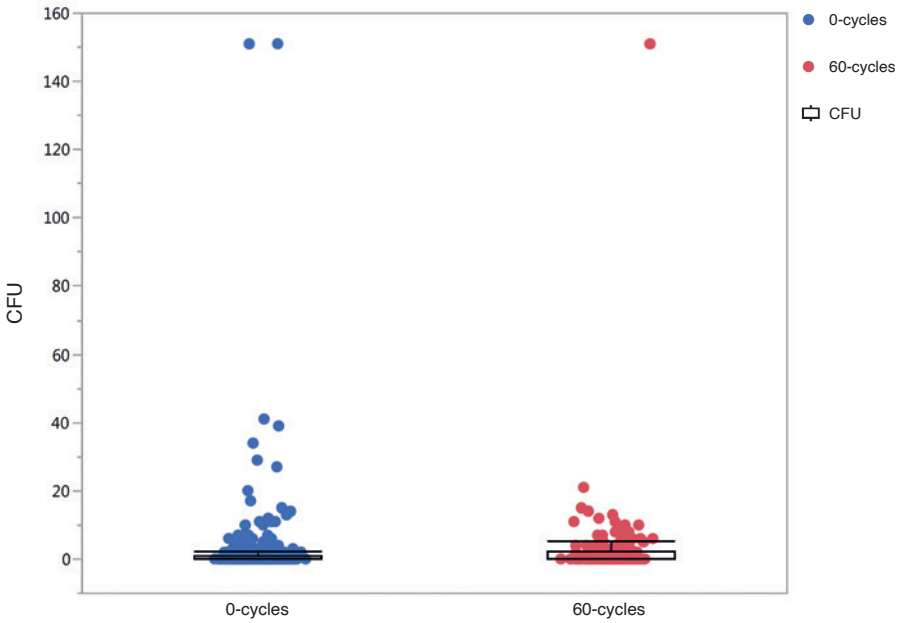

Figure 1: Microbial count analysis of the outside of the garment (output = colony forming unit) of the garment sets (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles) with overlaying box-plots.

The statistical analysis of the cleanroom garments with 0 and 60 or 40 cycles was carried out using a generalised linear model (model for negative binomial distribution) due to the high occurrence of 0 CFU events. In addition, a chi-squared test was used to check whether there was an association between the two ageing states. The determined bacterial counts were categorised according to the defined action limit for cleanroom garments into > 5 CFU (YES, contamination) and ≤ 5 CFU (NO, no contamination).

The results of the generalised linear model indicate that there is no significant difference between the two garment sets (0 and 60 resp. 40 cycles) ($p = 0.469 > 0.05$). Furthermore, no association between the two garment sets (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles) could be detected by means of a chi-squared test. Based on these results, it can be concluded that the garment sets with 0 and 60 or 40 cycles are microbiologically comparable.

3.3 Particle results

Using the BioTrak® system, the particle counts in the return air were recorded during the study of the cleanroom garments in the Body-Box. A distinction was made between the total airborne particles (total, T: analysed particle sizes ≥ 0.5 µm and ≥ 5 µm) and viable particles (V: analysed particle ≥ 1 µm).

The results of the particle measurements of particle sizes ≥ 0.5 µm and ≥ 5 µm were statistically separately analysed due to the different data distributions (particle sizes ≥ 0.5 µm log normal distribution, ≥ 5 µm negative binomial distribution).

Figure 2 shows the individual log transformed data with corresponding box-plot of the particle measurements with particle size ≥ 0.5 µm (0 and 60 resp. 40 washing, sterilisation and wearing cycles).

The data of the particle measurements with particle size ≥ 0.5 µm are subject to a log-normal distribution. For this reason, the data of the particle measurements (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles) were log transformed and subsequently compared with each other by means of a 2-sample T-test for significance ($p < 0.05$)).

No significant difference ($p = 0.1855 > 0.05$) was found between the particle measurements (particle size ≥ 0.5 µm) of the clothing with 0 and 60 resp. 40 decontamination, sterilisation and wearing cycles. As a result, the new garments are comparable to the garments with 60 or 40 decontamination, sterilisation and wearing cycles in terms of particle release (particle size ≥ 0.5 µm).

Figure 3 shows the individual data with the corresponding box-plot of the particle measurements of particle size ≥ 5 µm (0 and 60 resp. 40 cycles).

The statistical of the particle measurements with a particle size ≥ 5 µm (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles) was performed using a generalised linear model (model for negative binomial distribution) due to the high occurrence of 0 particle events. In addition, a chi-squared test was used to check whether there was an association between the particle measurements of the different ageing states. The determined particle counts were categorised into particles present (YES, contamination) and no particles present (NO, no contamination).

The results of the generalised linear model indicate that there is a significant difference between the two garment sets (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles) ($p < 0.05$).

In contrast, however, no association could be detected by means of a chi-squared test between the particle counts of particle size ≥ 5

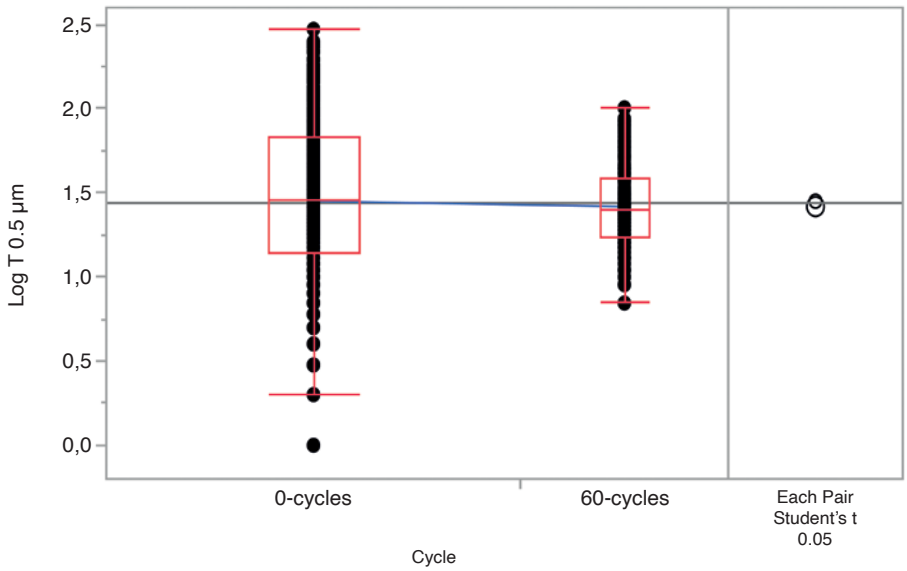

Figure 2: Log transformed data of the garments with 0 and 60 resp. 40 decontamination, sterilisation and wearing cycles with overlaying box-plots.

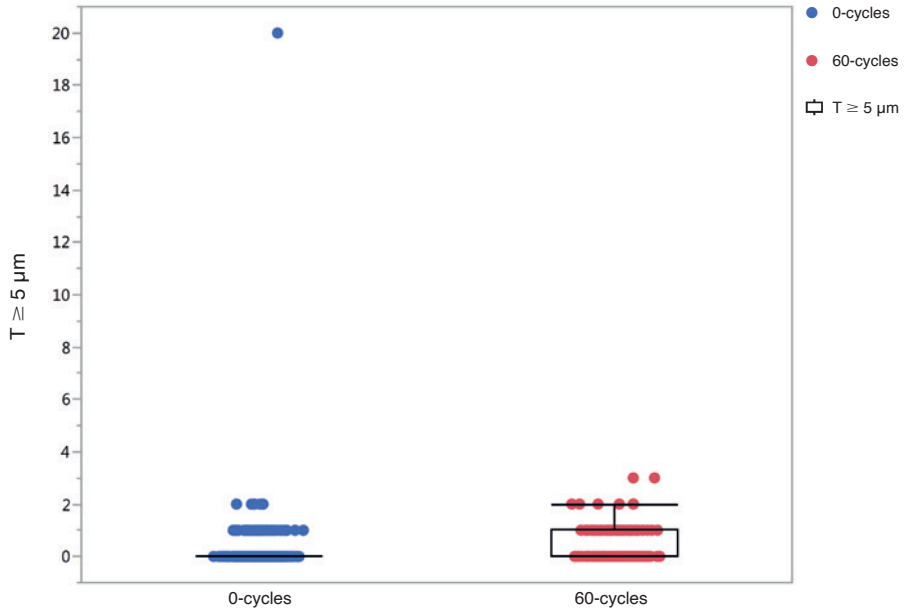

Figure 3: Particle measurements (particle size ≥ 5 µm) of the garment with 0 and 60 resp. 40 cycles with box-plots drawn in.

µm and the two garment sets (0 and 60 resp. 40 decontamination, sterilisation and wearing cycles). Based on these different results, it is not possible to make a clear statement regarding comparability on the basis of particle counts of particle size ≥ 5 µm. One possible reason for this ambiguous result could be that the studies of the cleanroom garment presented here were „only" carried out in a Body-Box, which does not represent a genuine cleanroom of zone A/B with associated personnel airlock, but rather the cleanroom itself guarantees an ISO Class 4, while the associated personnel airlock „only" represents an ISO Class 7. Thus, the particle counts can only be evaluated to a limited extent.

In the course of the generally relatively low particle counts recorded, only isolated airborne viable particles could be detected with the BioTrak® system. Due to the large number of measuring points without findings, no statistical analysis of the comparability of the garment sets of the different age states could be carried out here. Figure 4 shows the data for the age states 0 and 60 resp. 40 decontamination, sterilisation and wearing cycles as a bar chart. During the standing phases, no viable particles could be detected with the counter.

The analysis of the additionally prepared garment sets for the worst-case scenario could only be carried out with eight clothing sets. Individual parts had defects on wear parts, which is why no further measurements could be carried out with these. Therefore, the data from the worst-case scenario was not used for further comparison at this point. All in all, the 60 and 40 cycles could be confirmed as good end points with the safety buffer examined.

4. Discussion

Based on the data, it can be seen that the established garment system meets the requirements for use in cleanliness zones A/B and that the specified maximum decontamination and sterilisation cycles, including use of the cleanroom garment in between, proved to be adequate, since no statistically significant difference could be demonstrated between new cleanroom garments and cleanroom garments after maximum use. Thus, the garment system investigated here could be successfully requalified for the pharmaceutical producer. However, the results obtained in this study cannot be easily transferred to other – even if similar – cleanroom garment systems.

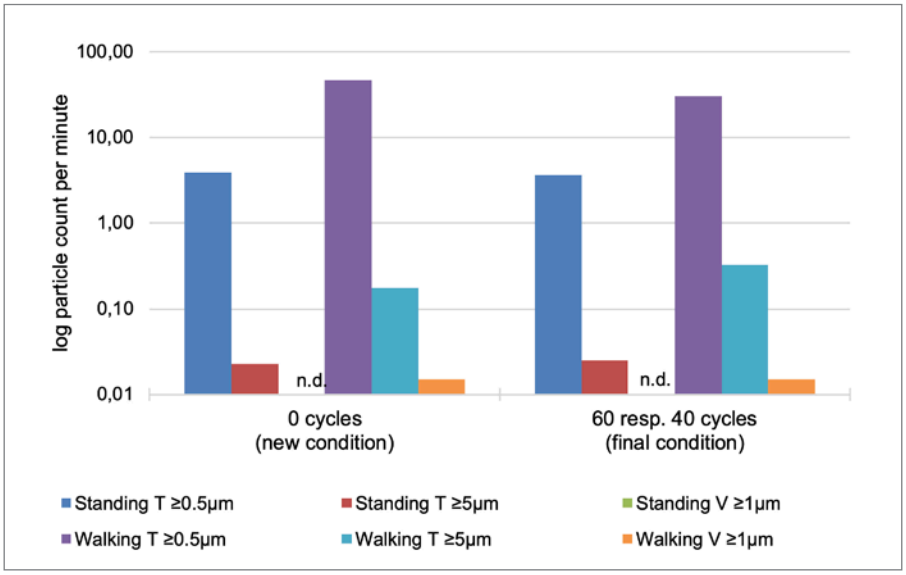

Figure 4: Airborne particles (T=total particle count, V=viable particle count as mean value per minute separated by movement phase (n.d. = not detected)

Different influencing factors can change the efficiency of a cleanroom garment system

1) Each cleanroom textile has its own characteristics, which may change differently with increasing number of washing, sterilisation and usage cycles.

2) Cleanroom compatible undergarments are a critical factor in the efficiency of a cleanroom garment system.

3) Each cleanroom laundry processes cleanroom garments under different conditions.

4) Mechanical stress from the wearers can vary greatly between different users and/or application processes.

5) Disinfectants or other additional influences during wearing affect the durability of the cleanroom garment.

6) The sterilisation process (duration, temperature, type) influences the durability and efficiency of the cleanroom garment.

7) Wearing time can vary greatly between different application processes.

8) The type and degree of contaminants to be removed can have an impact on the ageing characteristics of a cleanroom textile.

Consequently, each user should qualify/requalify its own cleanroom garment system according the relevant framework. Qualifying the efficiency of the cleanroom garment when new is not sufficient – qualifying the efficiency of the cleanroom garment with the maximum specified number of decontamination, sterilisation and wearing cycles should also be done.

In the case of an initial definition or a change of the cleanroom garment system, this is of course not yet possible at the beginning. Here, one can and must ultimately rely on empirical values of other users, on study results – such as those described here – and on the manufacturer's statements. If necessary, however, ageing phenomena can be simulated in a timely manner in order to be able to make a better risk assessment. With increasing use of the cleanroom garments, comparable tests, as described in this study, are then possible under the specific framework conditions – as part of the qualification process. Also essential for the interpretation and classification of results are the results and trend data of the company's own routine microbiological bacterial count determinations of the cleanroom and of the cleanroom garments (environmental monitoring). If we now combine these two elements:

- Qualification of the garment system, as well as
- Results and trend data of the microbiological bacterial count determinations of cleanroom and cleanroom garments during the use of cleanroom garments in cleanliness zones A/B,

this results in the contamination control strategy required in Annex 1 (recognise / define, evaluate and control possible contamination risks and optimise if necessary)

5. Forcast

The question for data on the qualification/requalification of cleanroom garment systems is likely to increase in the near future. Thus, as part of a follow-up study with another pharmaceutical producer, additional fabric properties were tested with regard to ageing phenomena. For this purpose, the recognised textile research institute DITV Denkendorf was commissioned with the corresponding tests. The tests included, on the one hand, the roughening behaviour and the tensile and tearing forces in the textile, as well as the air permeability and the particle retention capacity on the DITV filter test rig in accordance with VDI 3926.

The values determined in this new study using the Body-Box method in terms of release of airborne particles and viable contaminations tended to agree with the findings of the study discussed in this article. In particular, the data obtained at the textile research institute DITV Denkendorf showed - no significant decrease in particle retention (after 60 resp. 40 (for overboots) washing, sterilisation and wearing cycles) – only very slight deviations in the mechanical resilience of the fabric (after 60 washing, sterilisation and wearing cycles) and confirmed the statements on the defined washing, sterilisation and wearing cycles used here.

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