DUPONT™

Tyvek®

IsoClean®

EU GMP ANNEX 1

HOW TO VALIDATE PROTECTIVE CLEANROOM GARMENTS?

Introduction

The revision of the EU GMP Annex 1 for the manufacturing of sterile products was published and will be effective on the 25th of August, 2023. This document aims to provide valuable information regarding this revision and its new requirements.

Concisely, it requires that manufacturers of sterile products will apply the principles of Quality Risk Management (QRM) to the design and control of facilities, equipment (such as cleanroom garments), systems and procedures used for the manufacture of all sterile products.

Manufacturers of sterile products need to provide a proactive means of identifying, scientifically evaluating and controlling potential risks to quality and to ensure that microbial and particulate contamination is prevented in the final products. They must also implement a Contamination Control Strategy (CCS) across the facilities to define all critical control points and assess the effectiveness of all the controls (design, procedural, technical, and organisational) and monitoring measures employed to manage risks to medicinal product quality and safety.

QRM principles for cleanroom garments

The cleanroom garment assessment should be run under the QRM principles. Quality risk management starts with an analysis and understanding of all the risks to quality linked with cleanroom operators wearing cleanroom garments. A complete data-based analysis will allow to design certification, qualification, validation, and monitoring procedures which have quality built into them, thus being part of a holistic contamination control strategy. A risk analysis is needed to understand the contamination risks coming from operators wearing cleanroom garments. It has been scientifically demonstrated that operators represent the biggest source of contamination inside the cleanrooms and represent 75% of all contaminants[1]. This contamination is coming both from the operators themselves and from their cleanroom garments. The human contamination coming from the operators is due both to our human nature (an average person sheds 40,000 particles per minute and 10% of them carry micro-organisms) and human behaviour. The only measure to prevent that the particles generated by the operators will not contaminate the cleanroom are the cleanroom garments, they are the only barrier between the operator and the production environment. The 2022 EU GMP Annex 1 clearly points this out: "(the cleanroom garments should) retain particulates shed by the body".

It should not be neglected that the cleanroom garments themselves may be a source of contamination and this risk needs to be assessed too. For example, the material used for making the garments (non-woven for the single-use garments or woven for the reusables) can shed more or less particles depending on the nature of the fibers or filaments used, their resistance to abrasion or their construction as well as the effect of multiple wash-dry-sterilisation cycles. The trims (zipper, buttons, elastics or sewing threads) too may be a source of contamination. The design of the garment plays a role as well and should be evaluated. One detail which is often neglected is the packaging in which the cleanroom garments come, which could be a source of contamination too (i.e., paper-back bag vs. plastic bags).

[1] Ramstorp M., "Introduction to Contamination Control and Cleanroom Technology", Wiley VCH, 2000, Weinheim (Germany)

Main stages of the validation

M. Pavičić is owner and director of Pavičić Pharmaceutical Microbiology (PPM) and GOP - Innovations and an expert in GMP and sterility assurance. T. Wagner is the Global Director Regulatory & Standards for DuPont Healthcare

Once the risks have been evaluated, they should be, as far as possible, removed or replaced by technical or organisational means and the residual risks mitigated as much as possible using a validated cleanroom garment system. In their article "Risk & Science-Based Validation of Cleanroom Garments" from 2019, M. Pavičić and T. Wagner have offered a QRM based structured approach to validate cleanroom garments that meets EU general guidance on validation (GMP Annex 1519).[2] This approach has been integrated into ISO 13408-1 Aseptic processing of health care products — Part 1: General requirements approved in June 2023.

Quality Risk Management

Design Qualification

Adopted cleanroom clothing system meets URS by following GMP guidelines

- Risk assessment
- Fit for purpose solution
- Following GMP guidelines
- Meeting User Requirement Specifications
- Appropriate product attributes
- Testing of properties and characteristics
- Performance testing
- Stability testing
- Usability evaluation
- Sterilization validation

Installation Qualification

Installation of cleanroom clothing system

- Risk assessment
- All elements of cleanroom gowning system present
- Materials
- Facilities
- Logistics
- Certificates
- SOPs
- Operator training and qualification plan
- Risk controls implemented and working

Operational Qualification

Gowning, work, de-gowning as designed

Qualification of gowning and de-gowning concept

Qualification of the aseptic presentation of the garments

Garments adequate for work ?

- Adequate SOP's
- Adequate training and qualification plan
- Visual and microbiological assessment of gowning
- Work tasks can be executed properly
- Risk controls implemented and working
- Define worst case

Performance Qualification

Consistency and reliability

Risk assessment

Gowning qualification

- Visual and microbiological assessments
- Gowning qualification of all personnel

Qualification of the production environment & aseptic process validations

- Three replicates
- Worst case conditions
- Risk controls implemented and working

Monitoring and re-qualification program

High Impact on Quality → Quality by Design → Low Impact on Quality

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[2] Source: Pavičić M. & Wagner T., "Risk & Science-Based Validation of Cleanroom Garments", IVT Network 2019

Main stages of the validation

The EU GMP Annex 1 is calling for a scientific evaluation and control of all potential risks to quality. The evaluation of the cleanroom garments must also be based on scientific test data allowing to assess the performances of the garments as well as enabling a control of these performances over the lifetime of the garments. Simply relying on experience, visual checks and recommendations from the suppliers will not be enough any longer for the authorities. In their paper M. Pavičić and T. Wagner have suggested a series of criteria for validating cleanroom garments that can be measured, scientifically tested, and documented, thus meeting the expectations of the new EU GMP Annex 1 (see below).

Material Qualification	Performance Testing	Stability Testing	Usability Evaluation
Cleanroom garments	**Cleanroom garments**	**Single-Use garments**	**User scenarios**
• Fiber and particle shedding • Sterilization compatibility • Sterility assurance level • Particle filtration efficiency • Bacterial filtration efficiency • Porosity • Surface resistivity • Perforation resistance • Mechanical resistance • Protection against biological agents	• Body box testing • Helmke dum test	• Properties and characteristics at the end of shelf-life **Reusable garments** • Properties and characteristics after maximum number of laudering and sterilization cycles	• Transfer to classified storage area • Readability of labels • Easy opening of packaging • Aseptic unfolding of garments • Gowning • Donning additional accessories • (e.g., sterile gloves, face mask, goggles) • Work situations • Safety, biosafety • De-gowning
Packaging	**Sterile packaging**	**Sterile packaging**	**Packaging**
• Fiber and particle shedding • Bioburden • Penetration of commonly used disinfectants **Sterile packaging** • ISO 11607-1	• Influence of transport on integrity/ sterility (ISO 11607-1)	• Packaging integrity/sterility at the end of shelf-life (ISO 11607-1)	• Aseptic presentation of garments (multiple layers)

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Source: Pavičić M. & Wagner T., "Risk & Science-Based Validation of Cleanroom Garments", IVT Network 2019

Tests for material qualification

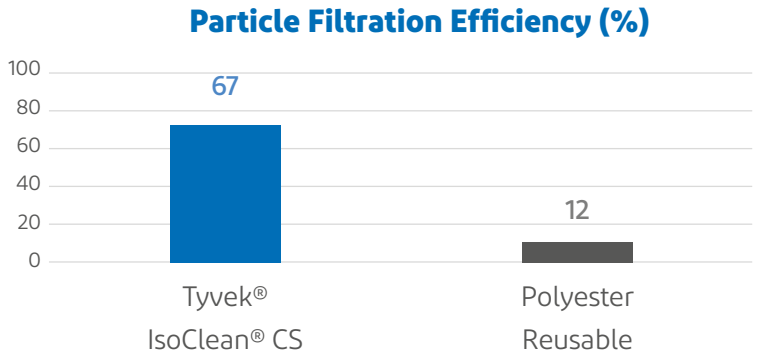
In this article here, some of these test methods will be explained with their advantages, as well as their disadvantages.

As stated above, the most important function of the cleanroom garments is to make sure to retain a maximum of the particles shed by the operators. Since the human being is constantly shedding particles and microorganisms, we must rely on the cleanroom garments to make sure that they stay inside the cleanroom garment and do not risk contaminating the cleanroom. It is therefore important to assess the filtration efficiencies of the garments, which are determined both by the structure of the material out of which the garments are made and the construction of the garments (i.e., seams and design). The former will be treated in this paragraph and the latter in the section on the garment qualification.

1) PARTICLE FILTRATION EFFICIENCY

The particle filtration efficiency (PFE) measures the filtration efficiency of the material used for cleanroom garments against the dry particles shed by the operators (i.e., skin flakes, even when stationary, people generate approximately 100,000 particles of 0.3 micron(µm) or greater).

The dry particle filtration of the materials depends mainly on the pore size of the fabric, the smaller the pore size, the higher the filtration efficiency. It may be assessed with test methods such as EN 143 (TSI 8130), which measure the filtration efficiency using salt particles having a diameter of 0.3 µm[3]. Since this is the smallest size of particles shed by humans and since the smallest size of particles used for the pharmaceutical cleanroom classification is 0.5 µm, this test is well suited for assessing the PFE of the materials, but since it assesses the fabrics only it cannot be used alone. For example, the PFE measured according to EN 143 for clean & sterile Tyvek® IsoClean® is >67% for particles bigger than 0.3µm while it is only 12% for a brand-new reusable cleanroom fabric made out of polyester monofilament.



Source: DuPont internal test

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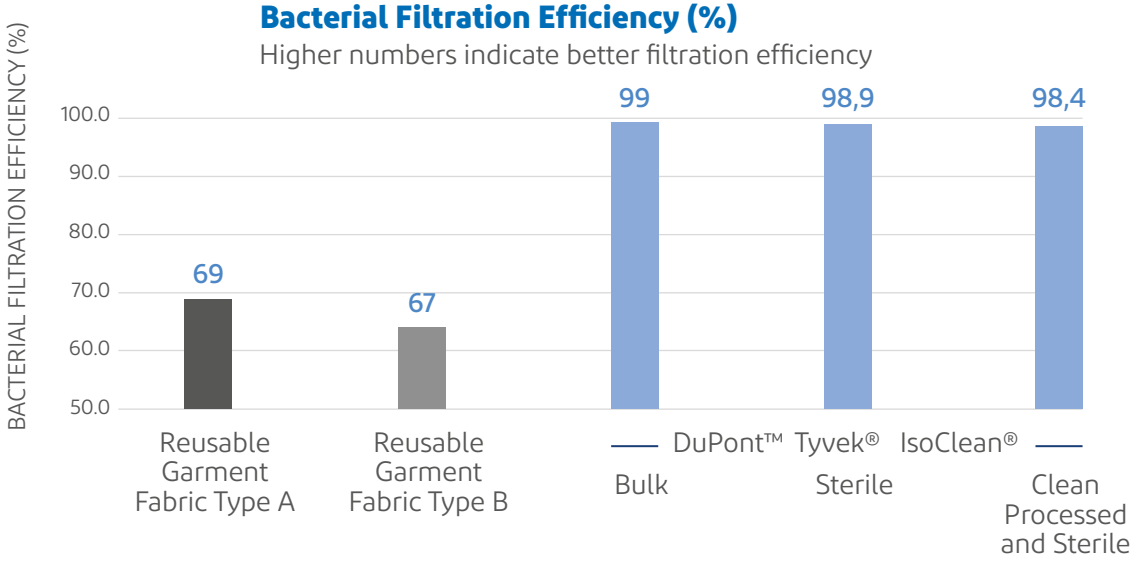
[3] Source : https://www.dupont.com/products/tyvek-isoclean-ic669b-option-0s.html

Tests for material qualification

In this article here, some of these test methods will be explained with their advantages, as well as their disadvantages.

2) BACTERIAL FILTRATION EFFICIENCY

The bacterial filtration efficiency (BFE) measures the filtration efficiency of the material used for cleanroom garments against bacteria shed by the operators. Humans release microorganisms through skin flakes (microbe-carrying particles) or sweat. The microbe-carrying particle filtration efficiency is again determined mainly by the pore size and may be assessed by the EN 143 test as well or by the ISO 22612, which measures the resistance to penetration by biologically contaminated solid particles. The liquid filtration efficiency is determined by the absorbency of the fabrics, the more liquid repellent a fabric is the higher its filtration efficiency. The ASTM F2101 standardized test method evaluates the bacterial filtration efficiency using a biological aerosol (Staphylococcus aureus) with a droplet size of 3 µm. Although this test was originally developed for medical face masks, it can also be used to evaluate other materials and is thus relevant for cleanroom clothing, as Staphylococcus aureus represents one of the highest sources of microbial contamination on the outer surface of cleanroom clothing, as demonstrated by Dr. Laurie Smith in her study[4]. While yielding pertinent results, this is also a material test only and therefore it should not be used as a sole assessment point. Below an overview of BFE test results.



*Results average of 10 measurements per fabric type from "as-received" garments
**Results as reported in SafeSPEC

[4] Source: Smith, L. Identification of Bacterial Isolates Recovered from the Surface of Cleanroom, Operators' Garments following Wear, 2022

Tests for material qualification

The particle retention performance is not only determined by the materials used, but also by the construction and the design of the cleanroom garments themselves. The IEST (Institute of Environmental Sciences and Technology) has developed test recomendations and methods for assessing the particle shedding and particle retention performances of cleanroom garments which are very useful for the qualification of cleanroom garment systems.

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The Helmke Drum test method as per IEST-RP – C003.4

The Helmke Drum is a rotating drum, with a rotating speed of 10 turns per minute, in which the cleanroom garments are being tumbled while a particle counter inside the drum is measuring the concentration of particles per minute for the sizes 0.3 µm and 0.5 µm. The results are then classified into 3 categories based on the number per size of particles released (see table below).

Category	Garment type	≥ 0.3µ	≥ 0.5µ
I	Coverall	< 2 000	< 1 200
II	Coverall	2 000 - 20 000	1 200 - 12 000
III	Coverall	20 000 - 20 0000	12 000 - 12 0000

This non-destructive test method is only measuring the particle release of cleanroom garments and is therefore quite widely used by cleanroom laundries to control the efficiency of their washing processes, but it has also been used by scientific studies to assess the particle release over time for cleanroom garments that are washed multiple times[5].

Since these studies have demonstrated that the particle release is increasing with each wash-dry-sterilisation cycle, the Helmke Drum test method may also be used for assessing the particle shedding over time in order to define the moment when the cleanroom garments need to be replaced. A visual inspection of the garments after the washing is inadequate to detect the degradation of the particle release of the cleanroom garments and the §7.11. *"Reusable garments (including eye coverings) should be replaced if damage is identified or at a set frequency that is determined during qualification studies. Damage to garments may not be identified by visual inspection alone, so the qualification should consider any necessary garment testing requirements. "*. However, the Helmke Drum test method does not provide data on the particle filtration efficiency of cleanroom garments, so should not be used as the unique qualification criteria.

The Helmke Drum test results should be documented in a certificate of compliance. Below an example:

DuPont Specialty Products USA, LLC
5401 Jefferson Davis Highway
Richmond, VA 23234 USA

DUPONT

CERTIFICATE OF COMPLIANCE

DuPont certifies that the Product and Lot Number below have been processed and released in accordance with DuPont Quality Management System procedures.

Representative samples of sterile and non-sterile Tyvek® IsoClean® Clean-Processed Garments are tested per IEST-RP-CC003.4 and meet Category I particle cleanliness (particles >=0.5 µm/minute) derived from the Helmke tumble test.

PRODUCT NAME:	IC501BWH000100MS
LOT NUMBER:	AHS2150153
DATE:	12/15/2021
PACKAGING IDENTIFICATION:	yangun-jiang(扬州厂)

A color change of the dot affixed below from yellow to red indicates that this lot has been gamma irradiated. DuPont certifies that the product number and lot numbers above have been manufactured and released in accordance to DuPont Quality Management Systems.

The minimum validation dose has been established in accordance with guidelines outlined in ANSI/AAMI/ISO 11137-1: Sterilization of Health Care Products - Requirements for Validation and Routine Control-Radiation Sterilization to provide a Sterility Assurance Level of 10⁻⁶.

Do not use if package integrity has been compromised.

Certificates of Sterility can be retrieved from myucpmemberservice@na.dupont.com or DuPont Customer Service @ 1-800-931-3456.

Approved By:
Quality Assurance

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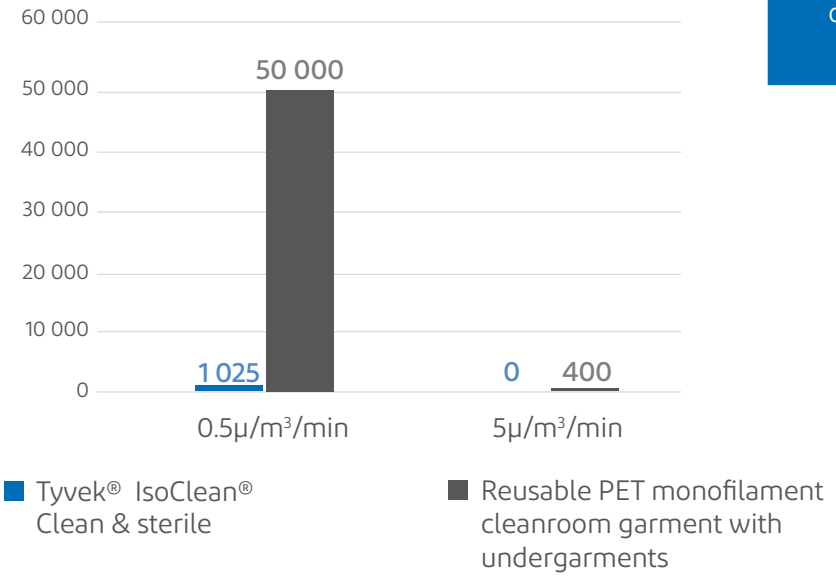
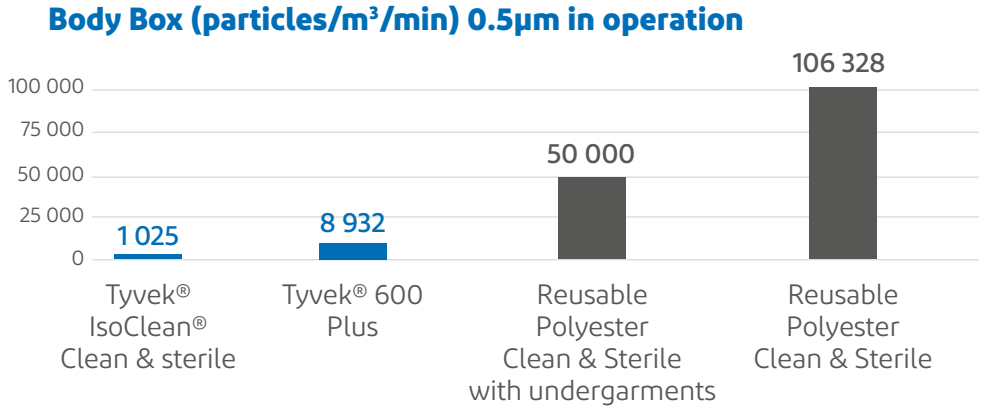
[5] For example: Romano F., Ljungqvist B., Reinmüller B., Gustén J. and Joppolo C.M., Performance test of technical cleanroom clothing systems, 2016 or Ljungqvist B. and Reinmüller B., Aseptic Production, Gowning Systems and Airborne Contaminants, 2004

The Body box test (IEST-RP-CC003.4)

This test is done inside a small cleanroom cabin in which an operator wearing a cleanroom garment system is performing a series of predefined movements during which the particles inside the body box are being measured and counted. This test best mimics real wearer conditions inside a cleanroom. It is measuring both the particle release of the cleanroom garments while they are being worn and the particle filtration efficiency of the garments. The lesser particles the garments shed and the better the particle filtration efficiency of the garments is, the lower the measured particles will be. Here some examples:





Source: Study by DuPont & C. Moschner, Kontaminationsquelle Mensch_2020_(01-2010).indd (dastex.de) for the reusable garments

Since this is a non-destructive test, it may also be used for assessing the performance of cleanroom garments which are washed multiple times in order to assess the moment when they need to be replaced. Various studies, such as those of Ljungqvist B. and Reinmüller B.[6] , show here also that the performance of reusable cleanroom garments is going down over time. As close to real work conditions the body box may be, it does have the drawback that the test is also measuring the particle release of the test persons without being able to distinguish which particles stem from the operator and which are released by the garment itself. As the study from Whyte et al[7]. shows, humans have a highly variable rate of particle shedding. Therefore, comparative tests are only meaningful if the same test person is used for running body box tests of different cleanroom garment systems or cleanroom garment that are more or less old. With the right test conditions, the body box is an excellent test for validating cleanroom garment systems.

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[6] Ljungqvist B. and Reinmüller B., Aseptic Production, Gowning Systems and Airborne Contaminants, 2004
[7] Whyte, W. and Hejab, M., Particle and microbial airborne dispersion from people, European Journal of Parenteral and Pharmaceutical Sciences, 12 (2). pp. 39-46. ISSN 0964-4679, 2007

Assessment of cleanroom garment sterility

In aseptic manufacturing (grades A/B) only sterile cleanroom garment systems may be used. It is expected that the sterilisation process is based on data, fully documented and is part of the contamination control strategy. Following a validated sterilisation process which can guarantee a sterility assurance level of 10^{-6} as per ANSI/AAMI/ISO 11137-1 is recommended to make sure the sterilization process is validated and controlled. The steriliser, manufacturer or laundry of the cleanroom garments should be able to provide a certificate of sterility. A simple certificate of irradiation or a protocol stating the temperature and duration of the autoclaving process is not sufficient anymore. Below an example of a Tyvek® IsoClean® certificate of sterility.

DUPONT

DuPont Specialty Products USA, LLC
5401 Jefferson Davis Highway
Richmond, VA 23234 USA

CERTIFICATE OF STERILITY

PRODUCT NAME:	DUPONT™ TYVEK® ISOCLEAN® HOOD/MASK
PRODUCT RELEASE DATE:	11/28/2021 12:00 AM
STERILIZATION RUN NUMBER:	M2102821B
EXPIRATION DATE:	NOV-2026
PART NUMBER:	IC689BWH000100TS
SIZE:	UN
STERILE LOT NUMBER:	AH52145166

GAMMA IRRADIATION STERILIZATION:

VALIDATED DOSE (KGY):	
MINIMUM SPECIFIED DOSE (KGY):	30.0
MAXIMUM SPECIFIED DOSE (KGY):	31.0
MINIMUM DELIVERED DOSE (KGY):	55.0
MAXIMUM DELIVERED DOSE (KGY):	34.0
	51.7

DUPONT CERTIFIES THAT THE LOT NUMBER ABOVE HAS BEEN RELEASED ACCORDING TO DUPONT INTERNAL SPECIFICATIONS. THE STERILITY ASSURANCE LEVEL (SAL) 10^{-6} WAS ESTABLISHED IN ACCORDANCE TO GUIDELINES OUTLINED IN ANSI/AAMI/ISO 11137.

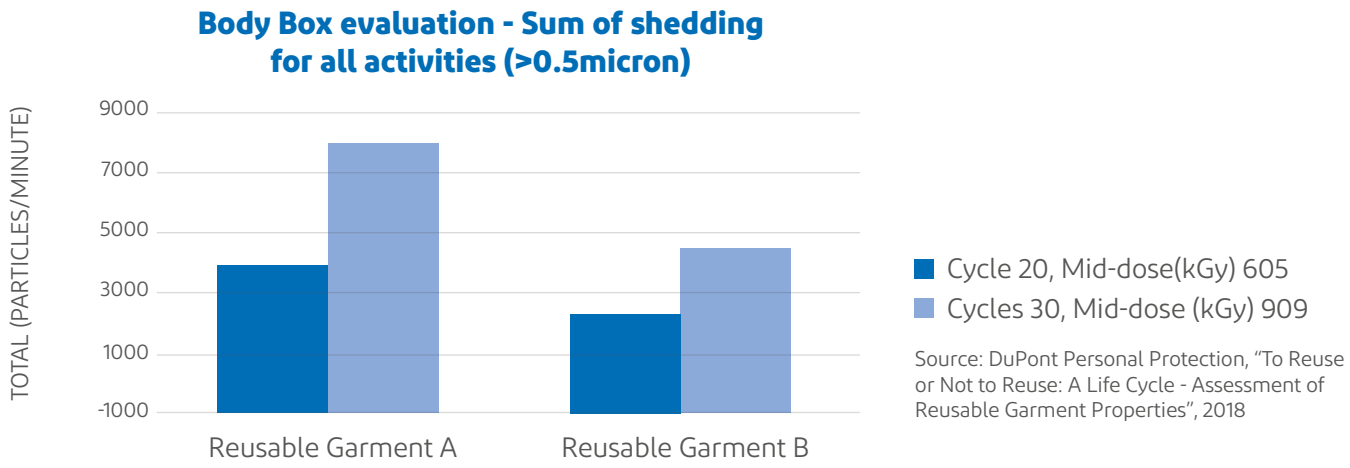
DO NOT USE IF PACKAGE INTEGRITY HAS BEEN COMPROMISED.

CERTIFICATES OF STERILITY CAN BE RETRIEVED AT MYCUSTOMERSERVICE.NA@DUPONT.COM OR CONTACT DUPONT CUSTOMER SERVICE AT 1-800-931-3456.

APPROVED BY:
QUALITY ASSURANCE

Stability Testing

In order to remain in control of the contamination risks at all times, it is important to ascertain that the cleanroom garments retain their performance levels each and every time they are worn inside the cleanroom. All the tests listed above may be used in the stability testing, which is easy for single use garments, such as the Tyvek® IsoClean® cleanroom apparel, it is more complex for reusable cleanroom garments. For single-use products randomly chosen garments that are close or past their shelf-life should be tested to see if they retain their performances over time. For reusable garments it is more complex because they washed, dried, and sterilized after each wear. As multiple studies have demonstrated, the wash-dry-sterilisation cycle has a detrimental effect on monofilament polyester reusable cleanroom garments and their performance levels deteriorate[8]. At DuPont we have run our own study using the body box test method and have demonstrated that after 20 wash-dry-sterilisation cycles the particle shedding has already increased significantly.



Source: DuPont Personal Protection, "To Reuse or Not to Reuse: A Life Cycle - Assessment of Reusable Garment Properties", 2018

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[8] For example: Romano F., Ljungqvist B., Reinmüller B., Gustén J. and Joppolo C.M., Performance test of technical cleanroom clothing systems, 2016 or Ljungqvist B. and Reinmüller B., Aseptic Production, Gowning Systems and Airborne Contaminants, 2004

Conclusion

Since operators represent the highest contamination risk inside pharmaceutical cleanrooms, the cleanroom garment systems are a critical part of the contamination control strategy. The new EU GMP Annex 1 is asking for a proactive, wholistic, risk-based and data-driven process validation. It is necessary that the selection of the cleanroom garment systems is based on scientific data and not only on experience, wearers' comfort and/or costs. Recognized testing should be used to assess the performances of cleanroom garment systems and to determine their end of life. This assessment should be part of a structured and well-documented approach which would fit well into the QRM based contamination control strategy and thus meet the expectations of the latest regulatory requirements.

References

1. Ramstorp M., "Introduction to Contamination Control and Cleanroom Technology", Wiley VCH, 2000, Weinheim (Germany)
2. Pavičić M. & Wagner T., "Risk & Science-Based Validation of Cleanroom Garments", IVT Network 2019
3. https://www.dupont.com/products/tyvek-isoclean-ic669b-option-0s.html
4. Smith, L. Identification of Bacterial Isolates Recovered from the Surface of Cleanroom, Operators' Garments following Wear, 2022
5. Romano F., Ljungqvist B., Reinmüller B., Gustén J. and Joppolo C.M., Performance test of technical cleanroom clothing systems, 2016
6. Ljungqvist B. and Reinmüller B., Aseptic Production, Gowning Systems and Airborne Contaminants, 2004
7. Whyte, W. and Hejab, M., Particle and microbial airborne dispersion from people, European Journal of Parenteral and Pharmaceutical Sciences, 12 (2). pp. 39-46. ISSN 0964-4679, 2007
8. DuPont Personal Protection, "To Reuse or Not to Reuse: A Life Cycle - Assessment of Reusable Garment Properties", 2018
9. ASTM, *"Standard Test Method for Evaluating the Bacterial Filtration Efficiency (BFE) of Medical Face Mask Materials, Using a Biological Aerosol of Staphylococcus aureus"*, https://www.astm.org/f2101-19.html
10. European Standards s.r.o. , *EN 143*, BS EN 143:2021 Respiratory protective devices. Particle filters. Requirements, testing, marking - European Standards (en-standard.eu)
11. European Standards s.r.o. , ISO 22612 - European Standards (en-standard.eu)

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