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STATISTICAL ANALYSIS OF CONTAMINATION IN CLASSIFIED CLEANROOMS AND OUTPUT INTERPRETATION: THE CASE OF A MEDICAL DEVICE MANUFACTURING COMPANY

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A me stesso, per aver avuto la pazienza di costruire, un giorno alla volta, ciò che oggi posso finalmente chiamare un traguardo.

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Abstract

The quality and safety of medical devices strongly depend on the control of manufacturing processes and environmental conditions within production facilities. In the biomedical sector, environmental and microbiological monitoring activities are essential to ensure compliance with contamination limits and maintain the required standards for controlled environments. Despite the large amount of data generated through these activities, environmental monitoring information is often distributed across multiple reports and validation protocol revisions, limiting its effective use for process evaluation and continuous improvement.

This thesis was carried out during a curricular internship at SIDAM S.r.l. within the Quality Assurance department and focused on the collection, organization, and statistical analysis of environmental monitoring and microbiological control data generated within the company's controlled environments. A major objective of the project was the creation of a centralized and standardized database capable of integrating historical information originating from different facilities, monitoring protocols, and validation protocol revisions. The database was developed to provide a structured and continuously updatable repository supporting future Quality Assurance activities and data-driven decision-making processes.

Once organized, the data were analyzed using the statistical software Minitab. Descriptive and inferential statistical methods were applied, including distribution analyses, hypothesis testing, control charts, and process capability analyses. These tools were used to evaluate process stability, identify sources of variability, compare different operational conditions, and assess the capability of monitored environments to maintain contamination levels within the required limits.

The results showed that all monitored environments remained compliant with the applicable particulate and microbiological contamination limits throughout the analyzed period. Production-related environments generally exhibited higher variability than support and transition areas due to the influence of manufacturing activities, personnel presence, and equipment installation. Process capability analyses confirmed that most monitored environments operated with adequate safety margins relative to specification limits, while also identifying areas characterized by lower capability and greater sensitivity to future variability.

Beyond the specific results obtained, the project demonstrated the value of applying statistical methodologies to environmental monitoring data and introduced a structured approach that

had not previously been systematically adopted within the company. The database developed during the study represents one of the main outcomes of the project and provides a foundation for future risk-based monitoring strategies, advanced statistical analyses, and the integration of environmental monitoring information with other quality-related data, such as non-conformities, complaints, deviations, and product quality controls. Consequently, the work contributes to strengthening data-driven Quality Assurance activities and supports the continuous improvement of environmental control systems within SIDAM S.r.l.

Introduction

In the medical device sector, product quality and safety represent fundamental requirements, as they are directly related to the protection of patient health. Companies operating in the biomedical field are therefore subject to a rigorous regulatory framework and must adopt quality management systems capable of ensuring control of production processes and compliance of products with the required standards.

In this context, the Quality Assurance (QA) department plays a key role in defining, implementing, and maintaining production standards while continuously improving process efficiency in order to guarantee product requirements in accordance with both company policies and international regulations. These activities are aimed at preventing defects before products are released onto the market, while also managing any non-conformities or complaints identified after commercialization.

A particularly important aspect of quality assurance concerns the control of environmental conditions within production areas. These environments, commonly referred to as Clean Rooms, must maintain particulate and microbiological contamination levels within predefined limits that determine their contamination classification according to international standards. Such limits become increasingly stringent depending on the type and classification of the medical devices manufactured within these controlled environments.

The state of the art in this field is represented by international regulations and standards, including those related to quality management systems and contamination-controlled environments. These standards provide the reference framework for the organization of manufacturing activities and for the implementation of environmental control systems within biomedical companies, ensuring that products are manufactured under conditions capable of guaranteeing their quality and safety.

This thesis was developed during a curricular internship at SIDAM S.r.l., a biomedical company specialized in the design, production, and commercialization of medical devices. The work was carried out within the Quality Assurance department with the objective of collecting, organizing, and analyzing data derived from periodic environmental monitoring activities and microbiological controls implemented by the company to ensure the quality of both manufacturing environments and marketed products.

The project initially involved the collection, verification, organization, and structuring of historical data originating from different company facilities, monitoring protocols, and protocol

revisions. This activity led to the creation of dedicated databases for environmental monitoring and product quality controls. Given the heterogeneous nature of the available information, a significant effort was required to standardize the data and create a consistent dataset suitable for subsequent statistical analyses.

Once organized, the data were analyzed using the statistical software Minitab through the application of descriptive and inferential statistical methods. Distribution analyses, hypothesis testing, control charts, and process capability analyses were employed to objectively evaluate process behavior, identify possible sources of variability, and assess the ability of monitored environments to maintain contamination levels within the required limits.

The analyses performed allowed the assessment of environmental and microbiological trends over time, as well as the comparison of different operational conditions and monitoring protocol revisions. Particular attention was dedicated to evaluating process variability and capability, providing objective information regarding the effectiveness of the environmental control system and the maintenance of the required quality standards.

The overall objective of the work was to establish a structured and sustainable framework for the management of environmental monitoring data within the company. Beyond the collection and organization of historical information, the project aimed to create a centralized database that can be continuously updated with future monitoring results, improving data traceability, accessibility, and long-term management.

A further objective was to introduce a statistical approach for the evaluation of environmental monitoring data, providing an objective method for identifying trends, assessing process performance, and highlighting environments characterized by higher variability or greater contamination risk. This type of analysis had not previously been systematically applied to the available environmental monitoring data and therefore represents an additional decision-support tool for Quality Assurance activities.

The developed database and analytical framework may also support future investigations aimed at correlating environmental monitoring results with product quality indicators, such as non-conformities, deviations, complaints, or other quality events. In this perspective, the project represents not only an assessment of historical environmental performance, but also a foundation for future risk-based monitoring strategies and continuous improvement initiatives within the company.

Furthermore, the activities carried out during this project represent a valuable starting point for future developments, including the implementation of automated data management systems and more advanced analytical tools. Dedicated software solutions could be developed to

automatically collect monitoring data, update databases, and facilitate data analysis, thereby supporting continuous process monitoring and Quality Assurance decision-making activities.

This thesis is organized as follows. Chapter 1 introduces the company context and describes the role of the Quality Assurance department. Chapter 2 presents the state of the art and the main regulatory references concerning quality management systems and controlled environments. Chapter 3 describes the materials, methods, and statistical approaches adopted throughout the study. Chapter 4 reports the results obtained from the environmental monitoring analyses. Chapter 5 discusses the findings, with particular focus on process variability and capability assessment. Finally, Chapter 6 summarizes the main conclusions of the work, highlights its limitations, and outlines potential future developments.

1 Company Overview and Quality system

1.1 SIDAM

SIDAM S.r.l. is a company operating in the biomedical sector, specialized in the design, production, and commercialization of medical devices. The company is located in the biomedical district of Mirandola (Modena, Italy), one of the world's leading hubs for the production of medical devices, characterized by a high concentration of technological and industrial expertise.

Founded in 1991, SIDAM has developed over time an organization capable of covering the entire product life cycle, from research and development to production, distribution, and after-sales support. Over the years, the company has strengthened its presence in the sector through integration activities and acquisitions of other manufacturing companies.[1]

SIDAM operates in a highly regulated environment, adopting quality management systems compliant with international standards to guarantee the safety and reliability of its medical devices. The company is certified according to the ISO 13485 standard and participates in the MDSAP (Medical Device Single Audit Program), which enables the harmonization of regulatory requirements across different countries through audits mutually recognized by the major regulatory agencies of the most highly regulated markets (United States, Canada, European Union, Australia, Japan, and Brazil). [1][5]

The product classes manufactured by SIDAM include drainage devices, infusion sets, radiology systems, disposable gynecology devices, prick test needles, nasogastric catheters, and urology devices, as well as components and accessories for specialized applications. In addition to manufacturing and marketing these products under its own CE-marked brand, SIDAM also operates as a Contract Manufacturer for products whose trademarks are owned by major customers.[1]

1.2 Production Facilities and Manufacturing Environments

SIDAM operates three manufacturing facilities located within the Mirandola biomedical district: two sites in Via Statale Sud and Via G. Bove in Mirandola, and one site in Via Maestri

del Lavoro in Medolla. Each facility is characterized by specific manufacturing activities and by an environmental configuration designed to meet the requirements of the production processes and the medical devices manufactured therein.[1]

From an organizational perspective, the facilities are divided into several operational areas characterized by different levels of environmental control. The most critical manufacturing activities are carried out within controlled environments (clean rooms), where particulate and microbiological contamination must be maintained within predefined limits in accordance with international standards. In SIDAM, the production clean rooms are classified as ISO 7 environments, while the supporting and transitional areas associated with them are generally classified as ISO 8.

To ensure effective contamination control, the clean rooms are supported by changing rooms, gowning areas, airlocks and pass boxes, which regulate the movement of personnel and materials between areas characterized by different contamination levels. The layout of these environments is specifically designed to minimize contamination risks through the separation of flows and the continuous control of environmental conditions.

The Via Statale Sud facility represents both the company headquarters and the main operational site, where activities including design, production, distribution and technical support are performed. Several classes of medical devices are manufactured at this site, including infusion systems, disposable medical devices and products for specialized applications. Production activities are carried out within a 200 m² ISO 7 clean room.

The Via G. Bove facility is mainly dedicated to the production of radiology infusion systems and infusion sets. Compared to the Via Statale Sud site, this facility is characterized by a higher degree of automation, with several manufacturing operations performed using robotic systems and automated equipment. Production activities are conducted within a 500 m² ISO 7 clean room.

The Medolla facility manufactures infusion and transfusion medical devices both under the SIDAM brand and on behalf of third parties. In addition, the site includes an AIFA-authorized clean room dedicated to secondary pharmaceutical packaging activities. Both clean rooms present within the Medolla facility are classified as ISO 7 environments.

The analyses presented in this thesis focus primarily on the Via Statale Sud and Via G. Bove facilities, which represent the sites where the environmental monitoring activities investigated in this work were performed.

1.3 Quality Assurance within the Company

Within SIDAM, the Quality Assurance (QA) function plays a key role in ensuring that production processes and products comply with regulatory requirements and quality standards applicable to the biomedical sector. QA provides the tools and methodologies necessary to monitor processes and minimize risk, through risk assessment activities, of placing unsafe or ineffective products on the market.

The Quality Assurance department is responsible for the definition, implementation, and monitoring of the Quality Management System (QMS), ensuring process traceability and reproducibility within validated variability limits, as well as document management and compliance with established operating procedures.

The company Quality Management System is described in the Quality Manual, which defines the organizational structure, responsibilities, and operational methods required to ensure compliance with applicable regulatory requirements. The Quality Manual represents the primary reference document for the management of company processes and includes information regarding the organizational structure, production facilities, certifications, and operating procedures adopted by the company.

Within this framework, data management plays a central role, as it enables process performance monitoring, the identification of potential critical issues, and the support of operational decision-making activities. The present work is positioned within this context, focusing on the organization and statistical analysis of data derived from environmental monitoring activities and microbiological controls.

1.4 Contamination and Controlled Environments

In the biomedical sector, contamination control represents a fundamental element in ensuring the quality and safety of medical devices. For this reason, the most critical manufacturing stages, involving device surfaces intended to come into direct or indirect contact with the patient, are carried out within controlled environments specifically designed to limit the presence of contaminants.

Clean rooms are enclosed environments maintained at a positive pressure relative to surrounding areas, where air is continuously filtered through High-Efficiency Particulate Air (HEPA) filters and environmental conditions are maintained under strict control. These environments are designed to minimize the concentration of airborne particles and microorganisms, thereby reducing the risk of product contamination during manufacturing activities.[3][6]

Contamination can generally be classified into two main categories: particulate contamination and microbiological contamination.

Particulate contamination consists of solid or liquid particles suspended in the air that may settle on surfaces, equipment, or directly on medical devices during the manufacturing process. The main sources of particulate contamination include operators, raw materials, machinery, packaging materials, and airflow systems. Personnel are considered one of the most significant sources of particulate contamination due to the continuous release of fibers, dust, skin particles, and residues originating from clothing and human activity.

The presence of particles within controlled environments represents a significant risk, as particulate matter may compromise product quality and act as a carrier for microorganisms. For this reason, particulate contamination must be maintained within limits defined by the UNI EN ISO 14644 standard, which classifies clean rooms into different ISO classes according to the maximum allowable airborne particle concentration.[3][6]

Alongside particulate contamination, microbiological contamination plays a critical role in contamination control programs. Microbiological contamination is caused by the presence of viable microorganisms such as bacteria, fungi, and yeasts. Unlike particulate contamination, microbiological contamination involves living organisms capable of surviving and proliferating under favorable environmental conditions, potentially compromising the safety and performance of medical devices.

One of the most important forms of microbiological contamination in controlled environments is airborne microbiological contamination, which refers to microorganisms suspended in the air. In many cases, microorganisms are associated with airborne particles that facilitate their transport and distribution throughout the clean room. The level of airborne microbiological contamination is influenced by several factors, including airflow patterns, manufacturing activities, material handling, and operator presence.

In addition to airborne contamination, surface microbiological contamination is also of particular relevance. This form of contamination refers to the presence of microorganisms on work surfaces, equipment, and materials located within controlled environments. Surface contamination is closely related to the materials used, the equipment installed, the manufacturing operations performed, and the personnel working within the clean room.[4][7][8]

To minimize contamination risks, several preventive measures are implemented, including cleaning and disinfection procedures for production areas and materials, specific gowning procedures for operators, and dedicated HVAC (Heating, Ventilation and Air Conditioning)

systems designed to maintain the required environmental conditions and air cleanliness levels.[3][4][6][7]

Microbiological monitoring, both surface and airborne, is performed using culture media specifically designed to detect and quantify microbial contamination. Airborne microbiological monitoring is generally carried out using active air sampling systems that collect a known volume of air and direct it onto appropriate culture media. Surface monitoring is typically performed using contact plates applied directly to critical surfaces. Following incubation under controlled conditions, microbial growth is evaluated by counting Colony Forming Units (CFU), which provide an indicator of the microbiological contamination level within the controlled environment.

Particular attention is also dedicated to microbiological monitoring of personnel, who are considered one of the primary sources of contamination within clean rooms. Even when appropriate personal protective equipment is used, operators may still contribute to the release of particles and microorganisms into the manufacturing environment. For this reason, microbiological monitoring is frequently extended to gloves, gowns, and garments used during production activities in order to evaluate the contribution of personnel to environmental contamination. [4][7][8]

To ensure adequate contamination control, periodic monitoring activities are performed on air, surfaces, and personnel. These activities allow verification of compliance with established acceptance limits, identification of potential deviations, and maintenance of the environmental conditions required for the manufacture of safe and effective medical devices.

1.5 Product Quality Control and Manufacturing Flow

Environmental monitoring activities represent only one component of the overall quality assurance strategy adopted by SIDAM. Alongside the control of manufacturing environments, particular attention is dedicated to the quality and microbiological safety of the medical devices produced within the company facilities. For this reason, specific controls are performed throughout the manufacturing process in order to verify compliance with regulatory requirements and to ensure the effectiveness of contamination control measures.

SIDAM manufactures several categories of medical devices, including infusion systems, administration sets, extension lines, radiology devices, needle-free connectors, and other disposable products intended for direct or indirect patient contact. Depending on the intended use of the device and the applicable regulatory requirements, different microbiological controls and sterilization processes are applied.

The main product families manufactured within the company, together with their applications and production sites, are summarized in Table 1.1.

Table 1.1 Main product families manufactured by SIDAM and corresponding production sites

Family	Subfamily	Use	Ster.	Statale	Bove
Infusion sets for drugs including chemoterapeutic	Administration set	Channeling of liquids for infusion or compounding, mixing and administration of drugs, including chemotherapeutic solutions (e.g. antiblastic, Taxol). These sets are intended to be directly or indirectly connected with infusion pumps.	EO	✓	
	Needle-free female valve				
	Preparation set with break-off system				
	Preparation set				
	Close male luer connector				
	Spike for bottle				
	Spike with balloon				
	Spike for large volumes				
	Extension set				
	Volumetric set pump adapter				
Sets for the preparation and administration of radiopharmaceuticals	Administration set	Channeling of liquids for infusion or compounding, mixing and infusion of radiopharmaceuticals, that may include different radioisotopes such as Lutetium 177 (Lu177), Yttrium 90 (Y90), Fluorine 18 (F18), Gallium 68 (Ga68), and Copper 63 (Cu63). These sets are intended to be directly or indirectly connected with infusion pumps.	EO/R	✓	✓
	Preparation set				
	Extension set				
Frii Power Motorized arthroscopic surgery device	Tool Body with blade/bur	Resection and removal of soft tissue (e.g., human joints) and bone, during arthroscopic surgery	EO	✓	
	Power internal unit & Charging station		/	✓	
Infusion sets for use with pump	Must kit Plus and Multi-patient administration set	Administration of contrast agents for CT or MR X-ray examinations	EO	✓	✓
	Set and lines for infusion working at low pressure	Administration of contrast agents, drug solutions or saline solutions for treatment of disease			
	Set and lines for infusion working at high pressure				
	Stopcock and manifolds	Administration of drugs and solutions for treatment of disease			
	Infusion and filtration set	Administrate drug solutions or saline solutions that require pre-filtration to the patient, either by infusion pump or by gravity for treatment of disease			
Drainage devices	Steel needle	Facilitate the insertion of a drainage device.	EO	✓	
	PVC drainage tube	Drainage of the thoracic cavity			
	PVC drainage tubes auxiliary				
	Thoracentesis/paracentesis set (with and without needle)	Drainage of abdominal cavity and drainage of the pleural cavity.			
Nutrivent	Single-Balloon naso-gastric catheter set	Monitor the esophageal pressure. Furthermore, the NutriVent catheter has all the functions of a normal feeding nasogastric catheter (nutrition	EO	✓	

		or sucking up liquids from the stomach).			
	Double-Balloon naso-gastric catheter set	Monitor the esophageal and/or gastric pressures.			
	Ventilator connecting line	Connect the Nutrivent catheter to a mechanical ventilator			
Dynamic esophageal stent	Esophageal stent	Treatment of benign esophageal stenosis. Using the nasogastric stent holder tube feeding the patient with enteral nutrition is possible	R	✓	
Infusion sets for use with gravity	Administration set	Infusion of contrast agent or saline solutions	EO	✓	
	Extension set				
	Spike				
Prick test	Prick test needle	Diagnosis of respiratory and food allergies by contact of the allergen to be tested with the subcutaneous area.	EO	✓	
Gravistop	Karmann-type cannula	Hysterosuction operations in gynocology, for aspiration endometrial tissue following voluntary termination of pregnancy; Gravistop are designed for joint use with suction pumps	EO	✓	
Optivent	Electromedical equipment	Used in combination with Nutrivent™ nasogastric catheter; management of the Nutrivent™ for the medical purpose of monitoring of disease.	/	✓	
Cyber Blade Morcellator	Tool Body with blade	morcellation and removal of dissected benign prostatic hyperplasia (BPH) tissue during endoscopic surgical procedures in urology	EO	✓	
	Power internal unit & Charging station		/	✓	
ALPItube	Excluding percutaneous ileostomy catheter	Protect colic anastomoses and in particular extraperitoneal colorectal anastomoses from enteric material	R	✓	
Xcope	Disposable kit	Used in combination with Virtual Reality Headset PICO 4 Ultra to isolate patient face and head from the direct contact with it	/	✓	
Warm-MB	Thermostatic cell	Heating and maintaining medical fluids (e.g. contrast media) at body temperature.	/	✓	
Pharmacy compounders	Preparation set	Preparation of radiopharmaceuticals	EO/R	✓	✓
Infusion sets (Subcontracting)	Administration set	Infusion of contrast agent or saline solutions	EO	✓	✓
Devices for the transport and storage of organs (Subcontracting)	Transport and storage kit	Transport and storage of organs	EO (Subcontractor)	✓	
	Accessory				
Set for the preparation and administration of radiopharmaceuticals (Subcontracting)	Cassette	Preparation and administration of radiopharmaceuticals for therapeutic and diagnostic purposes	/		✓

Among the most important microbiological controls is the bioburden test, which evaluates the population of viable microorganisms present on a product before sterilization. The

determination of bioburden is performed according to the requirements defined in UNI EN ISO 11737-1, which establishes microbiological methods for the estimation of the microbial population associated with healthcare products prior to sterilization [22].

Bioburden monitoring represents a fundamental tool for assessing the microbiological condition of the manufacturing process and verifying the effectiveness of contamination control measures implemented within the production environment. Excessive microbial contamination may compromise the effectiveness of sterilization and increase the risk of non-sterility of the final medical device.[4][22]

In addition to bioburden testing, bacterial endotoxin control is performed through the Limulus Amebocyte Lysate (LAL) test. Endotoxins originate primarily from the outer membrane of Gram-negative bacteria and may induce pyrogenic or inflammatory reactions even in the absence of viable microorganisms. For this reason, endotoxin monitoring is particularly important for medical devices intended for contact with biological fluids or the circulatory system.[4]

Following the manufacturing stages, medical devices undergo sterilization processes aimed at eliminating residual microbiological contamination.

In the biomedical sector, the most commonly adopted sterilization technologies include Ethylene Oxide (EtO) sterilization and irradiation sterilization.

Ethylene Oxide sterilization is particularly suitable for heat-sensitive devices and polymeric materials because it is performed at relatively low temperatures while maintaining the functional characteristics of the product. The sterilization process relies on the ability of the gaseous sterilizing agent to penetrate the packaging and inactivate microorganisms present on the device.[23]

Irradiation sterilization, on the other hand, uses ionizing radiation, typically gamma rays or electron beams, to damage the genetic material of microorganisms and prevent their replication. This technology is widely employed for industrially packaged disposable medical devices and allows sterilization to be performed without exposing the products to elevated temperatures.[24]

The manufacturing process adopted by SIDAM is organized through a structured flow designed to guarantee product traceability, quality control, and contamination prevention throughout all production stages as provided in Figure1.1

The process begins with the receipt of raw materials and components. Upon arrival, materials are initially placed in a quarantine area (Yellow Area), where they remain pending incoming quality control activities. Materials that do not meet the specified requirements are transferred to the Non-Conforming Material Area (Red Area), while approved materials are identified, labeled, and transferred to the Green Area dedicated to compliant materials available for production.

Following release, sampling activities are performed when required and the materials are transferred to the preparation area, where operators organize the components necessary for specific manufacturing orders. The prepared materials are subsequently introduced into the controlled production environment through dedicated material airlocks, where transfer procedures and container changes are carried out to maintain the cleanliness classification of the production areas.

Within the clean room, the main manufacturing operations are performed, including assembly activities, identification of semi-finished products, and primary packaging operations using pouches or blister packaging systems. These activities are conducted under controlled environmental conditions to minimize particulate and microbiological contamination.

At the end of the manufacturing process, products leave the clean room through a dedicated exit airlock and are transferred to the subsequent processing stages. Secondary packaging and labeling activities are then performed before the products are moved to dedicated storage areas awaiting sterilization.

Following sterilization, products return to the facility and are placed in quarantine pending final quality controls and release activities. Once conformity has been verified, finished products are transferred to the compliant finished product storage area and subsequently to the shipping area, from which they are distributed either to the final customer or, in the case of contract manufacturing activities, to the commissioning company.

This structured manufacturing flow allows SIDAM to ensure full traceability of materials and products, maintain compliance with quality requirements, and minimize contamination risks throughout all manufacturing, handling, sterilization, and distribution stages.

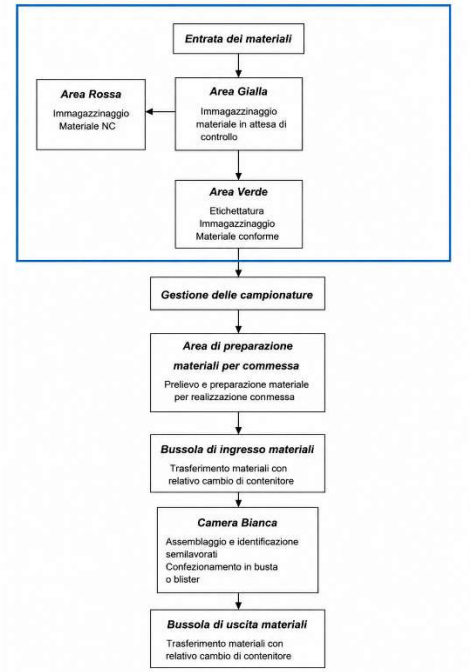


Figure 1.1 Material management and production workflow.

2 State of the Art

2.1 Regulatory Standards (ISO)

2.1.1 ISO 13485 – Quality Management System for Medical Devices

The International Organization for Standardization 13485 standard represents the main international reference for quality management systems in the medical device sector. The standard defines the requirements necessary for an organization to design, manufacture, install, and distribute medical devices while ensuring high levels of safety, reliability, and compliance with regulatory requirements.

The main objective of ISO 13485 is to ensure that all phases of the medical device life cycle are carried out according to controlled and documented procedures, minimizing the risk of defects or contamination that could compromise patient safety or product performance.

The standard is based on a risk-manaGEment approach and places particular emphasis on process traceability, procedure validation, document management, and non-conformity control. Furthermore, it requires companies to implement continuous monitoring systems together with corrective and preventive actions (CAPA) in order to maintain quality standards.

In the context of cleanrooms and controlled environments, ISO 13485 plays a fundamental role because it requires environmental conditions to be properly controlled during critical production phases. In particular, the standard establishes that processes capable of affecting the quality of the medical device must be carried out in monitored and validated environments, reducing the risk of particulate and microbiological contamination.

ISO 13485 does not directly define numerical limits regarding airborne contamination or microbiological presence; however, it requires these parameters to be controlled through the application of specific cleanroom standards, such as ISO 14644 for particulate contamination control and EN 17141 for microbiological monitoring.

Therefore, the adoption of ISO 13485 allows the integration of medical device quality requirements with environmental control strategies, ensuring regulatory compliance, product safety, and reproducibility of industrial processes.[5]

2.1.2 *ISO 14644 – Classification and Control of Airborne Particulate Contamination*

The International Organization for Standardization 14644 standard represents the main international reference for the classification and control of particulate contamination in cleanrooms and associated controlled environments. In particular, ISO 14644-1 defines the criteria used to classify air cleanliness according to the concentration of airborne particles present within the environment.
According to the standard, a cleanroom is defined as an environment in which the concentration of airborne particles is controlled and maintained within specified limits through ventilation and filtration systems designed to reduce the introduction, GEneration, and retention of contaminants.

The ISO classification system is based on the maximum allowable number of particles per cubic meter of air, considering different particle sizes ranging from 0.1 μm to 5 μm. The standard defines nine ISO classes, from ISO 1 to ISO 9, where lower ISO classes correspond to cleaner environments characterized by lower particulate concentrations.

To verify cleanroom compliance, ISO 14644-1 specifies dedicated sampling procedures using optical particle counters based on LSAPC technology (Light Scattering Airborne Particle Counter). The standard also establishes the minimum number of sampling locations, the air sample volume, and the procedures required for data acquisition and evaluation.

Cleanroom assessment can be performed under different operational conditions:

1. **as-built**, when the cleanroom is completed but without equipment or personnel;
2. **at-rest**, with equipment installed and operating but without personnel present;
3. **operational**, during normal cleanroom activity with personnel and production processes operating inside the environment.

Table 2.1 reports the maximum particulate concentration limits defined by ISO 14644-1 for the main ISO classes commonly used in biomedical and pharmaceutical controlled environments.

Table 2.1 Maximum allowable airborne particulate concentrations according to ISO 14644-1 (particles/m³). b = large sample volume required; c = not applicable; d = sampling limitations; e = sample collection limitations; f = macroparticle descriptor for ISO Class

ISO Class Number (N)	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
1	10	d	d	d	d	e
2	100	24[b]	10[b]	d	d	e
3	1 000	237	102	35[b]	d	e
4	10 000	2 370	1 020	352	83[b]	e
5	100 000	23 700	10 200	3 520	832	d,e,f
6	1 000 000	237 000	102 000	35 200	8 320	293
7	c	c	c	352 000	83 200	2 930
8	c	c	c	3 520 000	832 000	29 300
9[g]	c	c	c	35 200 000	8 320 000	293 000

As shown by the reported values, lower ISO class numbers correspond to higher levels of environmental cleanliness. In particular, ISO 5 and ISO 6 cleanrooms are commonly used in critical biomedical and pharmaceutical processes where the risk of product contamination must be minimized. ISO 7 and ISO 8 cleanrooms are instead generally employed in support areas or in environments characterized by less stringent contamination control requirements.[6]

2.1.3 *EN 17141 / ISO 14698 – Microbiological Contamination Control*

The European Committee for Standardization EN 17141 standard, together with International Organization for Standardization 14698, provides guidelines for microbiological contamination control in cleanrooms and associated controlled environments. These standards complement ISO 14644 by focusing not on particulate contamination, but on viable microorganisms that may compromise product quality, process reliability, or patient safety.

Microbiological contamination in controlled environments may originate from different sources, including personnel, air, surfaces, equipment, materials, and manufacturing activities. The most common contaminants include bacteria, fungi, yeasts, and spores. For this reason, microbiological monitoring represents a fundamental aspect in biomedical and pharmaceutical cleanrooms.

The main objective of EN 17141 and ISO 14698 is to establish a contamination control strategy based on risk assessment and continuous environmental monitoring. The standards require the identification of critical areas, the definition of alert and action levels, and the implementation of corrective and preventive actions in case of out-of-specification results.

Different microbiological sampling techniques can be used to evaluate the level of biocontamination within a cleanroom environment. The most commonly adopted methods include:

- active air sampling;

- passive air sampling using settle plates;
- surface sampling using contact plates;
- microbiological swab tests on critical surfaces and equipment.

Microbiological contamination is generally expressed in terms of CFU (Colony Forming Units), representing the number of viable microbial colonies detected after incubation. The obtained values may be reported as CFU/m³ for air samples or CFU/plate for passive monitoring methods.

Unlike ISO 14644, EN 17141 and ISO 14698 do not define mandatory universal microbiological limits. Microbiological contamination limits are generally established according to risk assessment, process criticality, product characteristics, and regulatory requirements. For this reason, environmental monitoring programs commonly adopt recommended alert and action levels based on GMP guidelines and contamination control strategies.

Table 2.2 reports examples of recommended microbiological limits commonly adopted in biomedical and pharmaceutical controlled environments during operational conditions.

Table 2.2 Recommended limits for microbiological contamination monitoring in controlled environments during medical device manufacturing. Limits are expressed as CFU (Colony Forming Units); b = limits applicable to aseptic processing of sterile medical devices

Category	Air sample (CFU/m³)	Settle plates (90 mm) CFU/4h	Contact plates (55 mm) CFU/plate	Glove print 5 fingers CFU/glove
1[b]	< 1	< 1	< 1	< 1
2[b]	10	5	5	5
3	100	50	25	N/A
4	200	100	50	N/A

As shown in the table, stricter environmental classes require significantly lower microbiological contamination levels. In particular, environments associated with highly critical or aseptic processes must maintain microbial concentrations lower than 1 CFU for air samples, settle plates, contact plates, and glove prints. Higher environmental categories allow progressively larger microbiological concentrations according to the contamination risk associated with the manufacturing process.

The standards also emphasize the importance of trend analysis, since environmental monitoring should not only identify contamination events but also detect progressive changes in

microbiological conditions over time. In the presence of abnormal results, investigations and corrective actions must be performed in order to restore controlled environmental conditions.

Therefore, EN 17141 and ISO 14698 play a fundamental role in ensuring microbiological safety in cleanrooms used for biomedical and pharmaceutical applications, supporting contamination prevention and regulatory compliance within controlled manufacturing environments.[7][8]

2.2 Application of Regulatory Standards at SIDAM

Within SIDAM, international standards related to controlled environments are implemented through a structured system of technical procedures, operational protocols, and validation activities aimed at ensuring the continuous control of cleanrooms and associated manufacturing processes.[2]

The application of ISO 13485, ISO 14644, and EN 17141 requirements is achieved through dedicated internal documentation, including quality procedures, environmental monitoring protocols, and validation plans used to ensure the compliance of production environments with applicable regulatory requirements.

Among the main adopted documents is the company technical procedure PT02 (“Cleanroom Validation and Control”), applied to the Via Statale Sud and Via G. Bove facilities. This procedure defines the operational methods used to verify the performance of cleanrooms and HVAC systems (Heating, Ventilation and Air Conditioning), with the aim of ensuring environmental conditions suitable for the manufacturing activities carried out within the controlled areas.

Validation is intended as a set of activities aimed at verifying the compliance of controlled environments, air treatment systems, and filtration systems with design specifications and applicable regulatory standards. Monitoring activities include particulate and microbiological contamination control, together with the verification of environmental parameters such as temperature, relative humidity, and differential pressure.[2]

The controlled environments within SIDAM facilities are mainly classified as ISO 7 and ISO 8 according to the UNI EN ISO 14644 standard. For particulate monitoring, particular attention is given to particles with a diameter of 0.5 μm, considered representative of the worst-case condition for environmental classification purposes. In addition to regulatory limits, operational alert and action levels are also defined in order to identify deviations before specification limits are exceeded.

Regarding microbiological monitoring, acceptable limits are defined within company protocols according to a risk assessment approach, in compliance with UNI EN 17141 and the ISO 14698 series. Microbiological controls are performed on air, surfaces, and operators in order to evaluate potential environmental contamination sources.[2][6][7][8]

Sampling activities are carried out using different methodologies. Particulate monitoring is performed through particle counters, while microbiological air sampling is conducted using active air sampling systems (SAS), which allow a known air volume to be directed onto dedicated culture media. Surface and operator microbiological monitoring is instead performed using contact plates applied to critical surfaces, gloves, hands, and garments.

Operational procedures, sampling locations, and monitoring frequencies are defined within dedicated company protocols. In the present work, protocol PL158 for the Via Statale Sud facility and protocol PL048 for the Via G. Bove facility were considered, analyzing different revisions considered representative of the evolution of the environmental monitoring system adopted by SIDAM.[2][12][13]

The considered protocol revisions highlight a progressive update of the environmental control strategy, including modifications related to sampling locations, cleanroom layouts, monitored parameters, microbiological limits, and revalidation activities. Sampling points are defined according to a risk assessment approach considering manufacturing activities, personnel flows, installed equipment, and potential contamination sources.

Environmental verifications are performed periodically, generally on a quarterly basis, through dedicated monitoring campaigns aimed at evaluating the compliance of controlled parameters. Obtained results are mainly assessed in terms of compliance with limits defined within company protocols, classifying each monitored parameter as either “within limits” or “out of limits”.[12][13]

Environmental monitoring activities are also strictly integrated with the company quality system for non-conformity and complaint management required by ISO 13485. Product-related issues identified on the market may require retrospective investigations aimed at verifying the environmental conditions present during manufacturing activities.

In particular, anomalies such as visible particle contamination on finished products may require a backward analysis of particulate monitoring data, microbiological controls, cleaning activities, and cleanroom operational conditions. Environmental monitoring data therefore represent an integral part of the investigation process and are used to support the identification of possible causes of non-conformities.[5][9][10]

Within SIDAM, the manaGEment of non-conformities, complaints, and related Corrective and Preventive Actions (CAPA) is formalized through dedicated company procedures, including procedures PG018 and PG013. These procedures define the methods for opening, managing, and closing investigations, including detailed corrective actions, preventive measures, and implementation timelines required to address detected issues.

Corrective actions may include, for example, increasing the frequency of environmental monitoring activities, revising cleaning procedures, performing additional HVAC system verifications, or introducing extraordinary monitoring campaigns, with the aim of minimizing the recurrence risk of identified problems.[9][10]

Alongside non-conformity and CAPA manaGEment, the company quality system also includes dedicated change control procedures aimed at managing modifications that may affect controlled environments, manufacturing processes, or monitoring systems.

Within SIDAM, these activities are manaGEd through company procedure PG05, which defines the operational methods for evaluating, approving, documenting, and verifying modifications introduced within the manufacturing system and cleanrooms. The procedure takes into account both regulatory requirements related to the different medical device markets served by the company and internal procedural constraints established by the ISO 13485-compliant quality system.
Each modification is formally documented, specifying responsibilities, planned activities, risk assessment evaluations, and acceptance criteria required for implementation. ChanGE control activities may involve modifications to cleanroom layouts, HVAC systems, environmental monitoring protocols, manufacturing processes, or product control specifications.
A significant example concerns the replacement of an Air Handling Unit (AHU) in an environment initially classified as ISO 8. Historical particulate monitoring data had already shown environmental performances compatible with ISO 7 limits. Following the installation of a more efficient AHU, the modification was manaGEd through the chanGE control process, including requalification activities and subsequent monitoring campaigns aimed at verifying compliance with ISO 7 environmental requirements.
The environmental reclassification was formally approved only after completion of the required verification activities and continuous compliance with acceptance criteria defined within qualification protocols. Similarly, change control procedures are also applied in cases involving the introduction of new equipment, modifications to manufacturing processes, or updates to product control specifications, ensuring full traceability of modifications and maintenance of regulatory compliance.[5][11]

2.3 Cleanroom Layout and Sampling Strategy

2.3.1 Via Bove Facility

The organization of controlled environments represents a fundamental aspect of contamination control within biomedical manufacturing processes. Cleanroom design, production area distribution, and the management of personnel and material flows are developed in order to minimize contamination risks and ensure controlled environmental conditions during manufacturing activities.

Within the SIDAM Via G. Bove facility, controlled environments are organized according to a hierarchical structure based on different cleanliness classifications. The main production areas are classified as ISO 7, while supporting and transition areas, such as changing rooms, pass boxes, and material transfer areas, are generally classified as ISO 8. This configuration allows a progressive separation between non-classified and critical production environments, reducing the risk of cross-contamination.

Figure 2.1 shows the global layout of the cleanroom and controlled areas of the Via G. Bove facility. The main ISO 7 cleanroom occupies the central part of the production area and is surrounded by supporting environments dedicated to material preparation, incoming control, maintenance activities, and auxiliary operations. Different material transfer systems, including pass boxes and material airlocks, are used to allow controlled transfer of components between areas with different cleanliness classifications without compromising cleanroom environmental conditions.

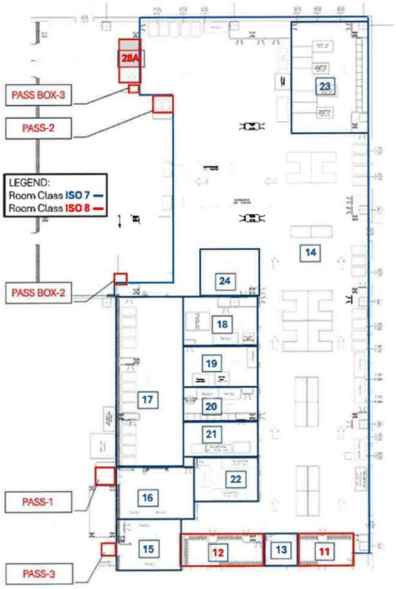


Figure 2.1 Layout of the controlled environments within the Via G. Bove facility.

The organization of the production spaces follows the operational workflow of the manufacturing activities carried out within the facility. Materials are initially introduced into receiving and inspection areas, then transferred to lot preparation areas, and finally introduced into the cleanroom through dedicated material airlocks. Inside the ISO 7 classified areas, the main assembly, processing, identification, and packaging activities of the medical devices are performed.

The different operational rooms are dedicated to specific manufacturing phases. In particular, the layout includes areas dedicated to component assembly, siliconization processes, tubing cutting, manufacturing cassette and incoming material handling. This subdivision allows separation of the different manufacturing activities, reducing contamination risks and ensuring a more efficient production workflow throughout the facility.

Table 2.3 summarizes the classification, surface area, and number of airborne particulate sampling points assigned to each controlled environment within the Via G. Bove facility. The design of controlled environments is also strictly connected to the definition of environmental monitoring strategies

Table 2.3 Airborne particulate sampling points used for environmental monitoring

ID	Nr. Area	Area	ISO Class	Surface (m²)	Nr. Sampling points
1	11	Men changing room	ISO 8	8.2	5
2	12	Women changing room	ISO 8	12.0	6
3	13	Airlock	ISO 7	5.2	3
4	14	Clean Room	ISO 7	376.8	25
5	15	Incoming Control	ISO 7	18.0	6
6	16	Lot preparation room	ISO 7	21.6	6
7	17	Warehouse preparation lot	ISO 7	57.8	12
8	18	Assembly sensor body US	ISO 7	16.8	6
9	19	Assembly membrane body	ISO 7	16.2	6
10	20	Siliconing room	ISO 7	9.8	5
11	21	Incoming materials	ISO 7	16.1	6
12	22	Tubing cutting room	ISO 7	14.7	6
13	23	Manufacturing cassette	ISO 7	41.3	10
14	24	Manufacturing meeting room	ISO 7	14.4	6
15	28A	Maintenance room	ISO 8	6.0	3
16	-	Pass Box 2	ISO 8	0.6	1
17	-	Pass Box 3	ISO 8	0.6	1
18	-	Pass 1	ISO 8	1.7	1
19	-	Pass 2	ISO 8	1.7	1
20	-	Pass 3	ISO 8	1.7	1

The design of controlled environments is also strictly connected to the definition of environmental monitoring strategies.

The distribution of airborne particulate sampling points, reported in Figure 2.2, is defined according to UNI EN ISO 14644 requirements, considering the surface area of the classified environments and the criticality level of the production processes. Areas characterized by larger surfaces and higher operational activity require a greater number of sampling points. For example, the main cleanroom (Room 14), characterized by an area of approximately 376 m², includes 25 sampling points for particulate monitoring, while smaller rooms require a lower number of control points.

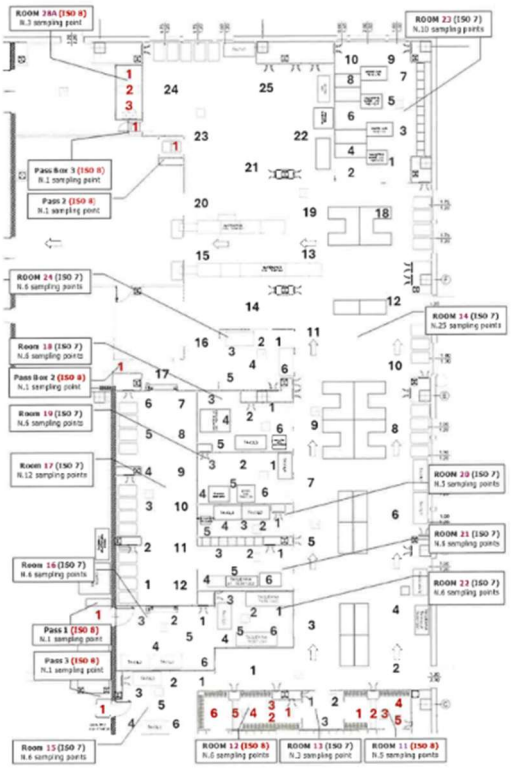


Figure 2.2 Airborne particulate sampling point distribution within the Via G. Bove cleanroom and associated controlled environments.

In addition to particulate monitoring, microbiological monitoring is also organized according to the production layout and the risk analysis associated with the different operational areas. The distribution of airborne microbiological sampling points, shown in Figure 2.3, highlights a higher concentration of monitoring activities within the most critical production areas and in locations characterized by elevated operator-material interaction.

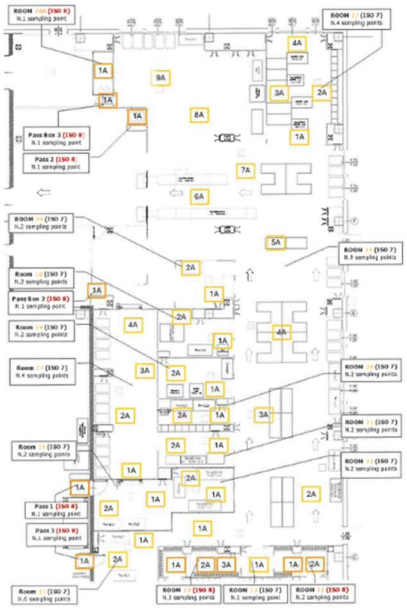


Figure 2.3 Airborne microbiological sampling point distribution within the Via G. Bove cleanroom and associated controlled environments.

Surface microbiological monitoring is instead performed by selecting specific surfaces considered critical from a contamination perspective. The controls are performed on working tables, machinery, sealing machines, containers, walls, floors, and access doors to classified areas. Particular attention is dedicated to surfaces directly involved in manufacturing activities and operator workflows.

Figure 2.4 shows the distribution of the surface microbiological sampling points adopted within the Via G. Bove facility. Surface microbiological monitoring is performed by selecting specific surfaces considered critical from a contamination perspective. The controls are performed on working tables, machinery, sealing machines, containers, walls, floors, and access doors to classified areas. Particular attention is dedicated to surfaces directly involved in manufacturing activities and operator workflows.

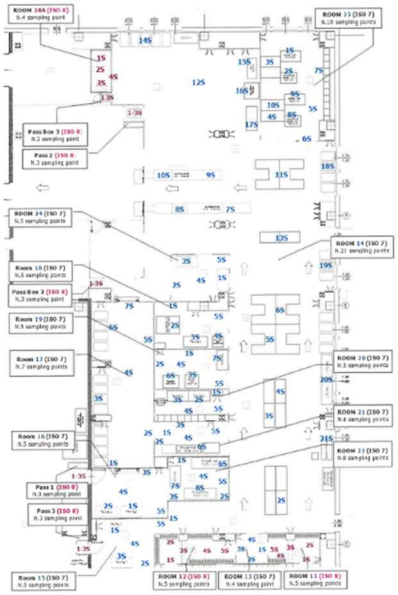


Figure 2.4 Layout of the Via G. Bove controlled environments showing the distribution of surface microbiological sampling points in ISO 7 and ISO 8 classified areas.

The overall organization of the controlled environments and monitoring strategies adopted within the Via G. Bove facility is therefore aimed at maintaining the environmental conditions required for medical device manufacturing, supporting Quality Assurance activities and continuous contamination control within the cleanroom environments.[12]

2.3.2 *Via Statale Facility*

Within the SIDAM Via Statale Sud facility, controlled environments are organized according to a configuration aimed at contamination control during the different assembly and packaging phases of medical device manufacturing. Also in this facility, the manaGEment of classified environments follows a hierarchical structure based on different cleanliness levels, with operational and supporting areas designed to guarantee flow separation and maintenance of the required environmental conditions.

Figure 2.5 illustrates the layout of the controlled environments within the Via Statale Sud facility together with the airborne particulate sampling locations adopted for environmental monitoring. The facility includes the main production cleanroom (Room 1), material inlet and outlet areas, airlock rooms, gowning and washing areas, and supporting rooms dedicated to quality control and material handling activities.

Figure 2.5 Layout of the Via Statale Sud controlled environments and distribution of airborne particulate sampling points.

The organization of the production spaces follows the operational flow of both materials and personnel. Components are introduced through the Inlet Materials Room (Room 2), then transferred into the main cleanroom for assembly and packaging activities, and finally directed toward the Outlet Materials Room (Room 3) for the subsequent phases of the manufacturing process. At the same time, personnel access classified areas through dedicated hand washing, gowning, and air lock rooms, minimizing contamination risks originating from external environments.

The different operational rooms are closely connected to the specific manufacturing activities carried out within the facility. In particular, areas dedicated to quality control (Q.C. Room), pressure testing, material balancing, and assembly activities are present. This subdivision allows separation of the different manufacturing processes while improving contamination and production flow control.

Table 2.4 summarizes the airborne particulate monitoring plan adopted within the Via Statale Sud facility, highlighting the number of sampling points assigned to each controlled environment according to its size and operational relevance.

Table 2.4 Number of airborne particulate sampling points assigned to the controlled environments within the Via Statale Sud facility, together with room classification and surface area.

Nr. ROOM	Area	ISO Class Design	Surface (m²)	Nr. Sampling points
1	CLEAN ROOM	ISO 8	164.00	22**
	PRESSURE TEST*	N.A.	N.A.	2
2	INLET MATERIALS ROOM	ISO 8	4.80	5
3	OUTLET MATERIALS ROOM	ISO 8	5.28	5
4	AIR LOCK WASHING	ISO 8	6.30	6
5	AIR LOCK DRESSING	ISO 8	6.60	6
6	BALANCE ROOM	ISO 8	25.0	7
7	AIR LOCK	ISO 8	14.0	6
8	ASSEMBLY ROOM - Q.C. ROOM	ISO 8	49.0	10

The design of the controlled environments is also strictly connected to environmental monitoring activities. The distribution of airborne particulate sampling points is defined according to room surface area and operational activity level. The main cleanroom includes the highest number of sampling points, while supporting areas characterized by smaller surfaces require a reduced number of environmental controls.

Microbiological monitoring is also organized according to the production layout and the risk analysis associated with the different operational areas. Airborne microbiological contamination limits, including specification, alert, and action levels, are summarized in Table 2.5. Microbiological specifications are expressed in CFU/m³ and are used to assess compliance with the environmental requirements established for the classified areas. The distribution of airborne microbiological sampling points is illustrated in Figure X. Sampling locations are concentrated within the main operational areas and in locations characterized by increased interaction between operators, materials, and medical devices.

Table 2.5 Airborne microbiological contamination limits (CFU/m³) for the controlled environments of the Via Statale Sud facility

Nr. ROOM	Area	Specification (CFU/m³)	Alert Levels (CFU/m³)	Action Levels (CFU/m³)
1	CLEAN ROOM	200	>160	>200
	PRESSURE TEST	10	>8	>10
2	INLET MATERIALS ROOM	200	>160	>200
3	OUTLET MATERIALS ROOM	200	>160	>200
4	AIR LOCK WASHING	200	>160	>200
5	AIR LOCK DRESSING	200	>160	>200
6	BALANCE ROOM	200	>160	>200
7	AIR LOCK	200	>160	>200
8	ASSEMBLY ROOM - Q.C. ROOM	200	>160	>200

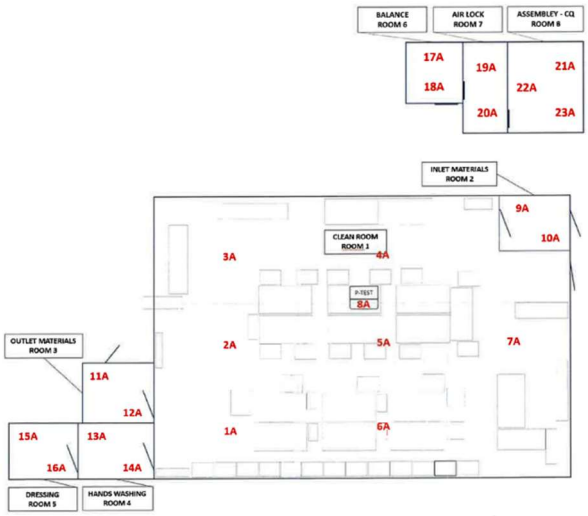


Figure 2.6 Layout of the Via Statale Sud controlled environments showing the distribution of airborne microbiological sampling points

Surface microbiological monitoring is performed by selecting surfaces considered critical from a contamination perspective. Table 2.6 and Table 2.7 reports the microbiological contamination limits adopted for surface monitoring together with the number of sampling points assigned to each controlled environment according to the environmental monitoring plan.

Table 2.6 Surface microbiological contamination limits (CFU/plate) of the controlled environments of the Via Statale Sud facility according to the environmental monitoring plan.

Surfaces	Specifications (CFU/Plate)	Alert Level (CFU/Plate)	Action Levels (CFU/Plate)
Surfaces except Floor	50	>40	>50
Floor	100	>80	>100

Table 2.7 Number of sampling points assigned to the controlled environments of the Via Statale Sud facility according to the environmental monitoring plan.

Nr. Area	Area	Surface (m^2)	Nr. of sampling points
1	CLEAN ROOM	164.0	10
2	INLET MATERIALS ROOM	4.80	5
3	OUTLET MATERIALS ROOM	5.28	5
4	AIR LOCK WASHING	6.30	5
5	AIR LOCK DRESSING	6.60	5
6	BALANCE ROOM	25.0	5
7	AIR LOCK	14.0	5
8	ASSEMBLY ROOM - Q.C. ROOM	49.0	6

The distribution of surface microbiological sampling points within the controlled environments is illustrated in Figure 2.7. Monitoring locations include working tables, walls, access doors, packaging components, machinery surfaces, and other surfaces directly interacting with materials and operators. These controls allow verification of the effectiveness of cleaning and disinfection procedures adopted within the classified environments.

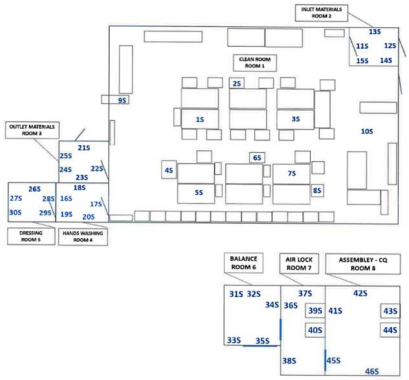


Figure 2.7 Layout of the Via Statale Sud controlled environments showing the distribution of surface microbiological sampling points.

The overall organization of the controlled environments within the Via Statale Sud facility is therefore aimed at maintaining the environmental conditions required for medical device manufacturing, supporting Quality Assurance activities and continuous contamination control within the classified production areas.[13]

3 Materials and Methods

3.1 Data collection and organization

The present study was developed through a retrospective collection of data related to microbiological and particulate environmental monitoring performed in three different production facilities: Via Bove, Via Statale Sud, and Medolla. The data were obtained through the analysis of official environmental monitoring reports periodically produced by the company as part of the control activities carried out in clean rooms and classified production areas.

For the Via Statale Sud and Medolla facilities, reports available from 2020 onwards were considered, while for the Via Bove facility only the reports available from 2023 were included. The use of different time intervals was related to document availability and to the presence of monitoring protocols that were more consistent with the objectives of the study.

During the preliminary phase of the work, a selection of the environmental monitoring protocols to be included in the analysis was performed. The selection was mainly based on revisions introducing significant modifications in monitoring criteria, sampling points, microbiological limits, or clean room management. The aim was to include protocols representative of the evolution of the environmental monitoring system adopted within the different facilities.

For the Via Bove facility, the selected protocol revisions were PL048 Rev.02, Rev.04, and Rev.05. These revisions were considered particularly relevant because they included updates related to the integration of microbiological and particulate monitoring, the introduction of new sampling points, and modifications to the clean room layout. Furthermore, the most recent revisions introduced laminar flow hood monitoring, environmental revalidation activities, and alignment with the requirements of UNI EN 17141:2021.

For the Via Statale Sud facility, the selected revisions were PL0158 Rev.02, Rev.03, Rev.04, and Rev.05. These revisions introduced substantial updates to the environmental monitoring plans, including modifications to the layouts of production areas, revisions of microbiological surface sampling points, and the introduction of specific microbiological limits for surfaces and operators' gloves. Some revisions also included updates related to environmental parameters such as temperature and humidity, together with modifications to microbiological classification criteria according to UNI EN 17141 requirements.

The protocols related to the Medolla facility were not described in detail because they were structured differently from the main protocols considered in this study and were less relevant for methodological comparison purposes.

Once the document collection phase was completed, all data underwent an organization and standardization process. Since the reports originated from different years, facilities, and protocol revisions, it was necessary to create a common structure capable of collecting all relevant information in a homogeneous manner.

This phase represented a fundamental step of the entire work, as it allowed the transformation of a large amount of heterogeneous data into a structured and easily searchable database, subsequently used for statistical analyses and comparative evaluations of environmental monitoring results.

3.2 Environment Database

Following the collection of reports and the selection of the protocols of interest, all environmental monitoring data were organized into a structured database. The creation of the database represented a crucial phase of the study, since the original data originated from different facilities, years, and protocol revisions that were not completely uniform. Therefore, a standardization process was required to create a single coherent structure capable of allowing homogeneous comparisons between results obtained in different production contexts. The overall organization of the Environmental Monitoring Database is illustrated in Figure 3.1, while the complete description of the fields included in the database is reported in Table 3.1.

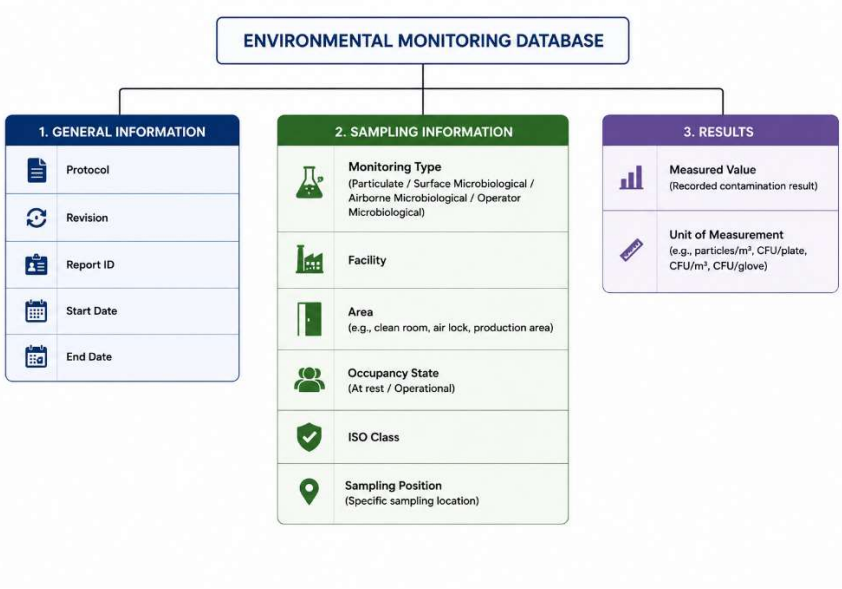


Figure 3.1 Logical structure of the Environmental Monitoring Database

Table 3.1 Structure of the Environmental Monitoring Database. The table summarizes all fields included in the environmental monitoring database, describing the information recorded for each monitoring activity, including protocol identification, sampling condition, monitored environment and analytical results.

Field	Description
Protocol	Monitoring protocol applied during the reference period.
Revision	Revision of the monitoring protocol.
Report ID	Identification number of the original monitoring report.
Start Date	Start date of the monitoring activity.
End Date	End date of the monitoring activity.
Monitoring Type	Type of environmental monitoring performed (particulate, surface microbiological, airborne microbiological, or operator microbiological monitoring).
Facility	Production facility where monitoring was performed.
Area	Specific monitored area (e.g., clean room, air lock, production area).
Occupancy State	Environmental condition during sampling (at rest or operational).
ISO Class	ISO classification of the monitored area.
Sampling Position	Specific sampling location within the monitored area.
Measured Value	Recorded contamination result.
Unit of Measurement	Unit associated with the measured value (e.g., particles/m^3, CFU/plate, CFU/m^3, CFU/glove).

The database included both particulate and microbiological environmental monitoring data. In particular, it comprised particulate monitoring of classified areas, microbiological surface monitoring, airborne microbiological monitoring, and operator microbiological monitoring, providing a comprehensive overview of the environmental contamination status of the clean rooms and production areas analyzed in this study.

As summarized in Table 3.1, each monitoring record included information related to the applied protocol and its revision, the monitoring period, the report identification number, the monitoring category, the production facility, the monitored area, the occupancy state during sampling, the ISO classification of the environment, the sampling position, and the measured contamination value together with its corresponding unit of measurement.

Particular attention was paid to the occupancy state of the monitored environment. Data were classified as either at rest, when no operators were present in the clean room, or operational, corresponding to routine manufacturing activities performed in the presence of personnel. This distinction is particularly relevant because operators represent one of the major sources of both microbiological and particulate contamination in controlled environments.

The standardized organization of all collected variables ensured complete traceability of each monitoring activity while facilitating homogeneous comparisons between different production facilities, monitored areas, protocol revisions, operational conditions, and monitoring categories. Consequently, the resulting database constituted the basis for all subsequent statistical analyses presented in this study.

3.3 Product Database

In addition to the Environmental Monitoring Database, a second database was created to collect and organize the results of LAL and bioburden analyses performed on production samples. The data were retrospectively collected from company reports available from 2020 onwards for the different production facilities included in this study. Since the analytical results originated from different reports, production batches, and manufacturing sites, a standardization process was required to create a homogeneous dataset suitable for subsequent statistical and comparative analyses.

The overall organization of the Product Database is illustrated in Figure 3.2, while the complete description of the fields included in the database is reported in Table 3.2.

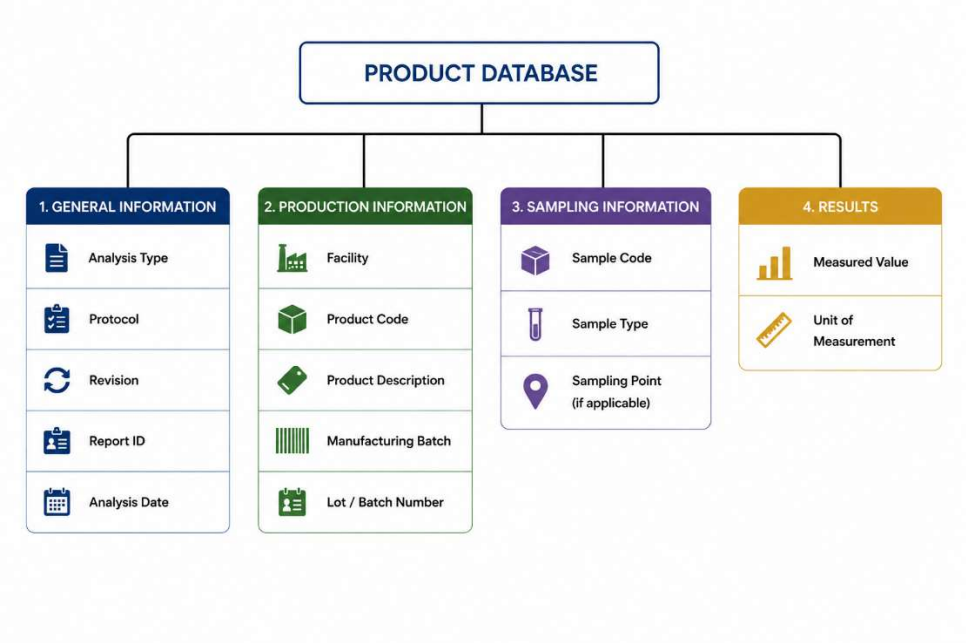


Figure 3.2 Logical structure of the Product Database.

Table 3.2 Structure of the Product Database. The table summarizes all fields included in the product database, describing the information recorded for LAL and bioburden analyses, including sample identification, production details, and analytical results.

Field	Description
Report ID	Identification number of the original analytical report.
Start Date	Start date of the analysis.
End Date	End date of the analysis.
Analysis Type	Type of analysis performed (LAL or bioburden).
Facility	Production facility where the sample originated.
Product REF	Product reference code.
Lot Number	Manufacturing batch identification number.
Quantity Tested	Quantity of sample analyzed.
Measured Value	Recorded analytical result.
Unit of Measurement	Unit associated with the measured value (e.g., EU/device, CFU/device, CFU/g).

The database included both bioburden analyses, aimed at evaluating the total microbial load present on production samples before sterilization, and LAL analyses, performed to determine the presence of bacterial endotoxins using the Limulus Amebocyte Lysate assay. The inclusion of both analytical methods provided a comprehensive overview of the microbiological quality and endotoxin contamination of the investigated products.

As summarized in Table 3.2, each analytical record included information related to the report identification number, the analysis period, the type of analysis performed, the production facility, the product reference code, the manufacturing lot number, the quantity tested, and the measured analytical result together with its corresponding unit of measurement.

The standardized organization of these variables ensured complete traceability of each analytical result while facilitating homogeneous comparisons between different production facilities, products, manufacturing batches, and analysis types. Consequently, the Product Database constituted the basis for all subsequent statistical analyses aimed at evaluating microbiological and endotoxin contamination trends and identifying potential recurring criticalities associated with specific products or production sites.

3.4 Statistical analysis of environmental data

Once the data collection, organization, and standardization phases had been completed, the study focused on the statistical analysis of environmental monitoring data collected from classified production areas. The analyses were performed using Minitab software, which is widely employed in industrial and pharmaceutical environments for statistical process control, variability assessment, and process performance evaluation.

The primary objective of the statistical analysis was to evaluate environmental contamination trends over time, identify possible differences associated with successive monitoring protocol revisions, and assess the stability of environmental control processes in the investigated production facilities.

Because particulate and microbiological monitoring data exhibit different statistical characteristics, two distinct analytical approaches were adopted. Particulate monitoring datasets generally consisted of a larger number of observations and showed distributions that could be adequately modeled using theoretical probability distributions. Consequently, these datasets were analyzed through distribution identification, descriptive statistics, histograms, probability plots, Individual Control Charts (I-Charts), process capability analysis (Cpk), and inferential statistical tests, including one-way Analysis of Variance (ANOVA). Whenever significant differences between protocol revisions were identified, two-sample t-tests were subsequently performed to compare consecutive revisions.

In contrast, microbiological monitoring datasets were characterized by a limited number of observations, a high frequency of zero values, and markedly non-normal distributions. For these reasons, distribution identification and process capability analyses were not considered appropriate. Instead, the analysis focused on descriptive statistical methods and graphical tools, including histograms and boxplots, which allowed the evaluation of data variability and the comparison of microbiological contamination levels between protocol revisions and monitored environments.

Overall, the adopted statistical workflow enabled the characterization of data distribution, process variability, environmental stability, and the presence of significant differences between monitoring conditions. The combination of descriptive, graphical, and inferential statistical methods provided a comprehensive assessment of environmental monitoring performance and supported the identification of contamination trends, variability sources, and potential criticalities within the analyzed production environments.

3.4.1 *Description of statistical tools*

Several statistical and graphical tools were used for the analysis of environmental data, selected according to the characteristics of the datasets and the objectives of the study. These tools allowed the evaluation of data distribution, process variability, and the presence of significant differences between groups.

Control charts were used to evaluate process stability over time and identify potential sources of special-cause variation. Individual Control Charts (I-Charts) were applied to particulate monitoring data, since observations were analyzed individually without subgrouping. In these charts, the x-axis represents the chronological sequence of observations, while the y-axis reports the measured contamination values. The charts allow the distinction between common-cause variability, inherent to the process, and special-cause variability associated with unusual events or process deviations. Consequently, they provide a useful tool for assessing environmental process stability and detecting potential out-of-control conditions.[14] Each chart includes a central line representing the process mean together with upper and lower control limits (UCL and LCL), as illustrated in Figure 3.3. These limits define the expected range of common-cause variability, whereas observations outside the control limits or exhibiting non-random patterns may indicate the presence of special causes requiring further investigation.

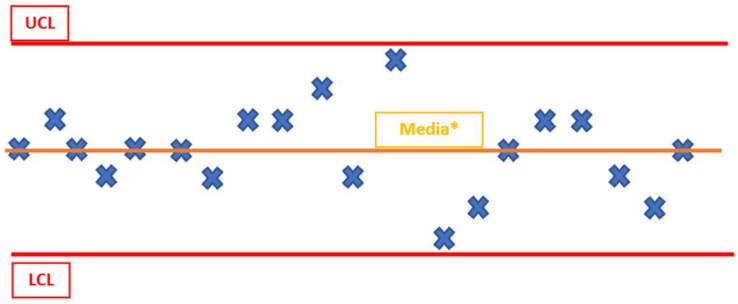


Figure 3.3 Schematic representation of an Individual Control Chart (I-Chart) showing the process mean, upper control limit (UCL), lower control limit (LCL), and individual observations over time.

An example of an Individual Control Chart obtained during the statistical analysis is reported in Figure 3.4.

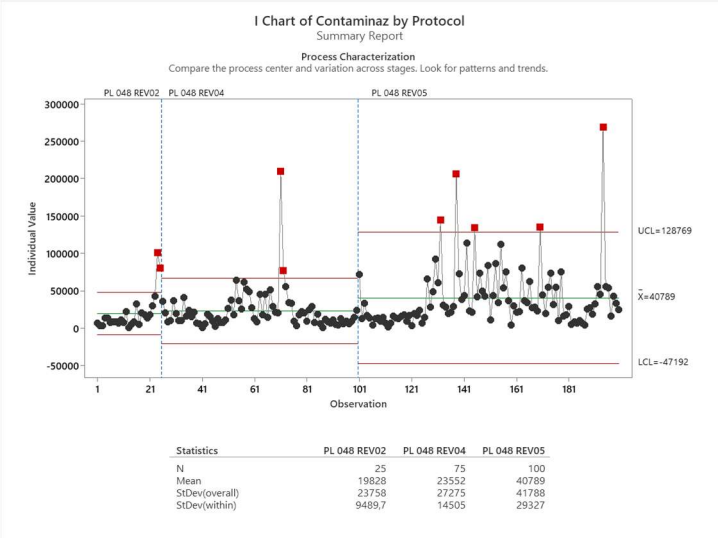


Figure 3.4 Example of I- Chart

In this example, the central green line represents the process mean, while the red lines indicate the upper (UCL) and lower (LCL) control limits. The vertical dashed blue lines separate the different protocol revisions. Individual observations are plotted in chronological order, and the red markers identify outliers exceeding the control limits. This type of chart allows the evaluation of process stability over time and facilitates the identification of unusual events requiring further investigation.

Histograms were used to represent data distribution and visualize the frequency with which specific values occurred within the sample. The shape of the histogram allows the observation of possible asymmetries, data concentration, or deviations from theoretical distributions. An example of a histogram is shown in Figure 3.5. Histograms provide a graphical representation of the frequency distribution of observations by grouping data into intervals, allowing a preliminary evaluation of distribution shape, central tendency, and variability.

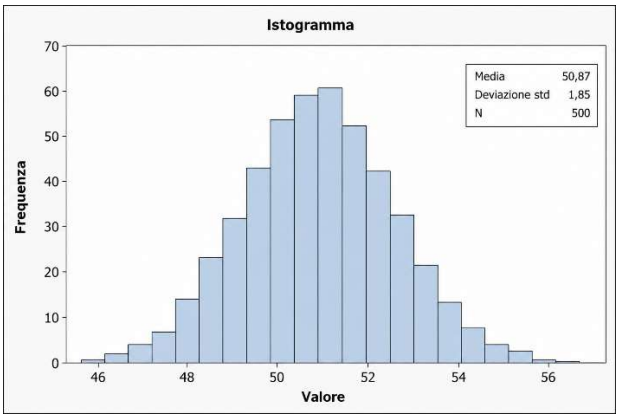


Figure 3.5 Example of a histogram showing the frequency distribution of observations grouped into classes.

An example of a histogram generated during the statistical analysis is presented in Figure 3.6.

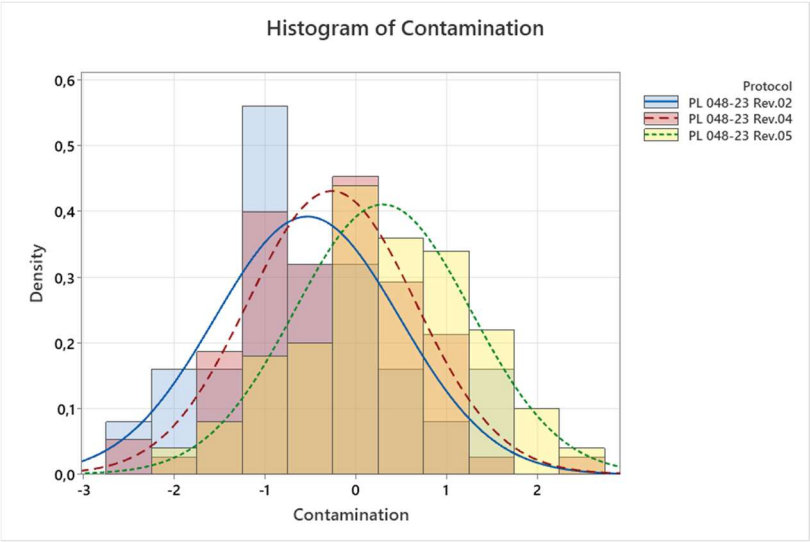


Figure 3.6 Example of istogram of different protocol revisions

Figure 3.6 illustrates an example of the histogram adopted for the analysis of particulate contamination data. The observations are grouped into frequency classes, while the fitted probability density curves represent the theoretical distributions identified for each protocol revision. By superimposing the histograms of the different revisions, the graph allows a direct comparison of the distribution shape, central tendency, and variability of particulate contamination over time. Differences in the position, spread, and overlap of the distributions provide a preliminary indication of changes in the environmental monitoring process before performing inferential statistical analyses.

Another graphical tool used in this study was the boxplot, a quartile-based representation that provides a synthetic description of data distribution. The boxplot allows visualization of the median, the dispersion of the central 50% of observations, and the possible presence of outliers, making it particularly useful for comparing different groups of data.[15] An example of a boxplot is shown in Figure 3.7. Boxplots provide a graphical summary of data distribution by displaying the median, quartiles, overall dispersion, and potential outliers. They are particularly useful for comparing different datasets and identifying extreme observations that may influence subsequent statistical analyses.

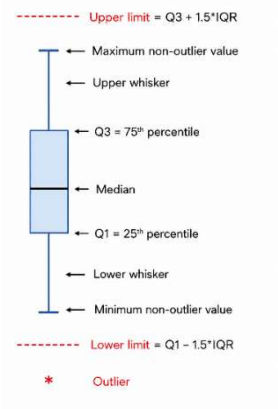


Figure 3.7 Example of a boxplot showing the median, interquartile range (IQR), whiskers, and potential outliers.

An example of a boxplot used for microbiological monitoring data is shown in Figure 3.8.

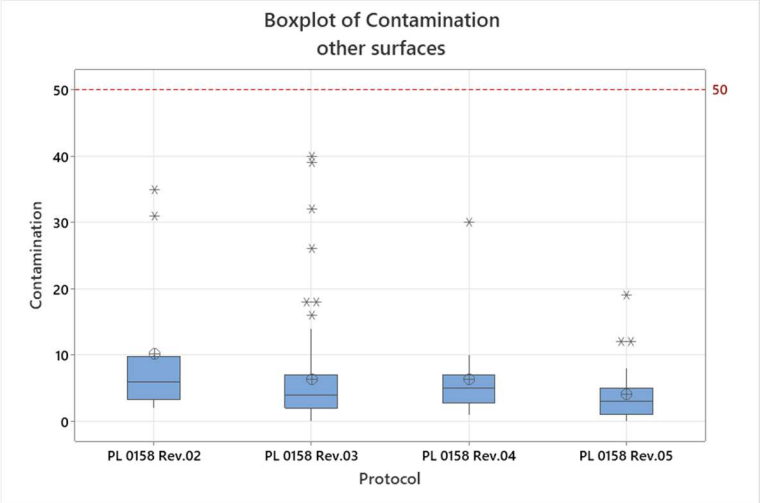


Figure 3.8 Example of Boxplot

Figure 3.8 illustrates a boxplot comparing surface microbiological contamination across different protocol revisions. The horizontal line inside each box represents the median, while the lower and upper edges correspond to the first and third quartiles, respectively. The whiskers describe the dispersion of the observations, whereas isolated points indicate potential outliers. This graphical representation facilitates the comparison of central tendency, variability, and extreme observations among the investigated revisions.

A fundamental step of the statistical analysis involved evaluating data distribution. In particular, the possibility of describing the datasets through a normal distribution was assessed, since this condition is required for the application of several parametric statistical methodologies.

The normal, or Gaussian, distribution represents a theoretical model in which values are symmetrically distributed around the mean. To identify the most appropriate statistical model, different distributions were compared, including normal and lognormal distributions, as well as Box-Cox and Johnson transformations. The model showing the best fit to the data was selected according to the obtained p-value.[17]

The goodness-of-fit was additionally evaluated through the Anderson–Darling (AD) statistic, where lower values indicate a better agreement between the experimental data and the theoretical distribution. The AD statistic was interpreted together with the corresponding p-value to identify the most appropriate distribution model.

A schematic representation of the normal distribution is shown in Figure 3.9. The normal distribution is characterized by symmetry around the mean and is fully described by two

parameters: the mean and the standard deviation. It is commonly used as a reference model for assessing data normality and comparing alternative distribution models.

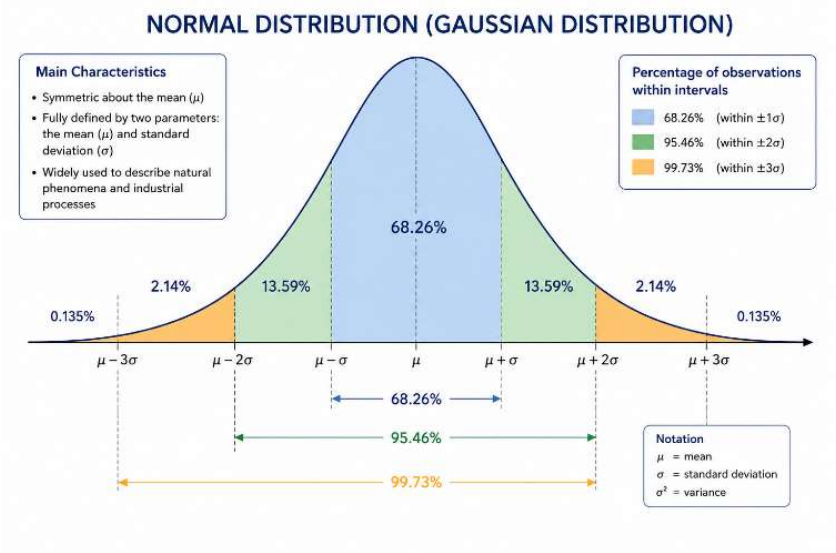


Figure 3.9 Schematic representation of the normal distribution, showing the percentage of observations expected within one, two, and three standard deviations from the mean.

To evaluate data dispersion around the mean, the standard deviation was also considered. This statistical parameter measures process variability: low standard deviation values indicate data concentrated around the mean, whereas higher values indicate greater variability among observations.[16]

Among the statistical tools applied, process capability analysis was also performed using the Process Capability Index (Cpk) and the Process Performance Index (Ppk). Both indices evaluate the ability of a process to comply with predefined specification limits by considering process variability and the position of the process mean relative to those limits.

The Cpk index is calculated using the within-process standard deviation and reflects the potential capability of a stable process under controlled conditions.

Conversely, the Ppk index is based on the overall standard deviation and therefore represents the actual long-term process performance, including both within- and between-subgroup variability.

A conceptual representation of process capability analysis is shown in Figure 3.10

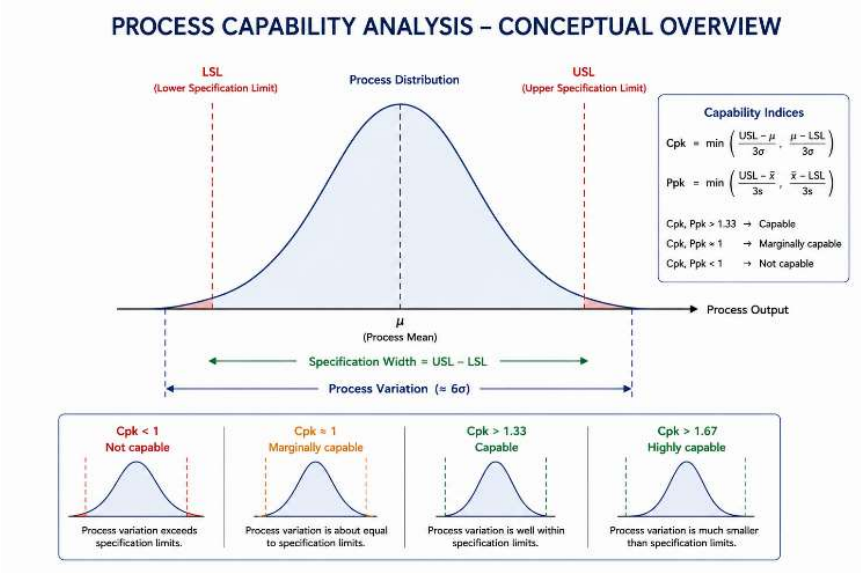


Figure 3.10 Conceptual representation of process capability analysis, showing the relationship between process variability and specification limits.

As reported in the literature, Cpk and Ppk values lower than 1 indicate a non-capable process, values between 1 and 1.33 indicate a marginally acceptable process, whereas values greater than 1.33 identify a capable process that is generally considered able to satisfy the required specifications consistently.[19][20]

In this study, Ppk was considered the primary capability indicator, since it reflects the overall variability of the environmental monitoring process across the different protocol revisions.

An example of a process capability analysis is presented in Figure 3.11.

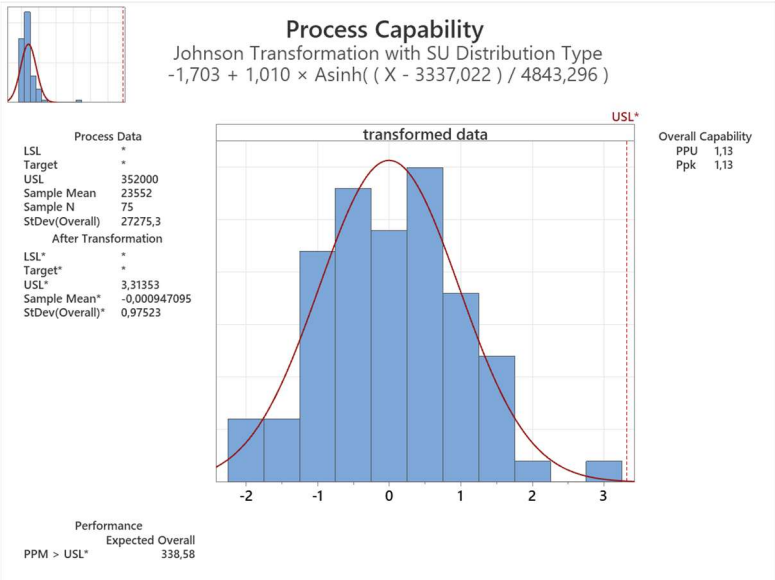


Figure 3.11 Example of Process Capability

Figure 3.11 presents an example of the process capability analysis performed on particulate contamination measurements. The histogram represents the distribution of the transformed data, while the fitted curve corresponds to the theoretical Johnson distribution. The red dashed vertical line identifies the upper specification limit (USL). The capability analysis reports both the Cpk and Ppk indices, which quantify the capability and overall performance of the process relative to the specification limit. The expected proportion of nonconforming observations is also expressed as parts per million (PPM > USL).

Finally, inferential statistical analyses were performed to evaluate the presence of significant differences between groups of observations. In particular, Analysis of Variance (ANOVA) was used to compare the means of multiple groups and determine whether the observed differences were statistically significant. When necessary, t-tests were subsequently applied for pairwise comparisons between groups.[21]

An example of the graphical output generated by one-way Analysis of Variance (ANOVA) is reported in Figure 3.12.

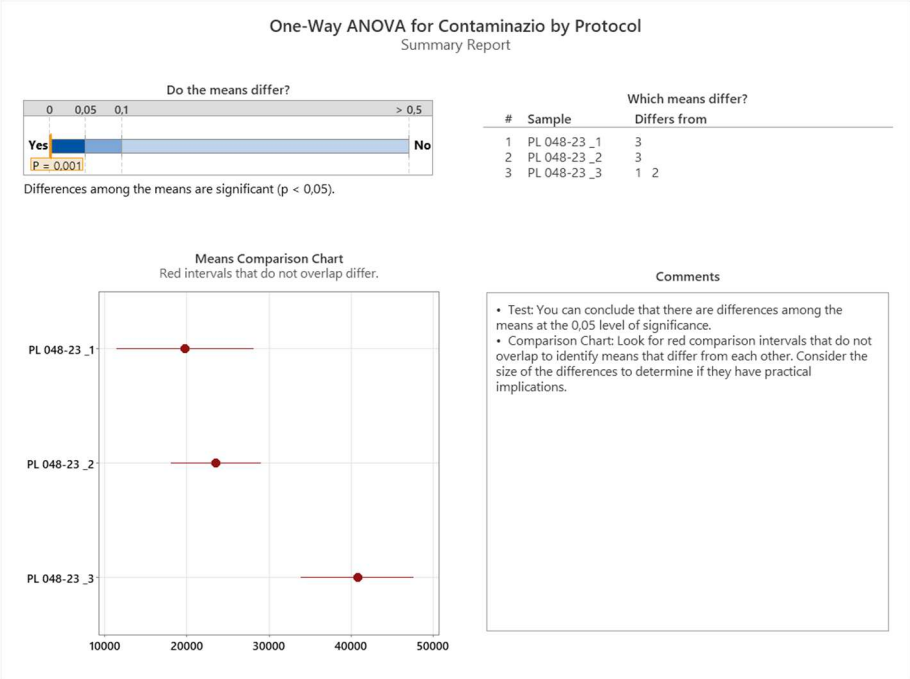


Figure 3.12 Example of Anova Test

Figure 3.11 illustrates the graphical output of a one-way ANOVA comparing particulate contamination measurements collected during different protocol revisions. The p-value indicates whether statistically significant differences exist among the compared groups, whereas the Means Comparison Chart identifies which group means differ significantly from one another. This analysis allows the evaluation of the effect of protocol revisions on environmental contamination levels.

4 Results

4.1 Data Distribution

In this subsection, the distribution analysis of environmental monitoring data collected from the selected monitored environments is presented. The most suitable theoretical distribution was identified using the Distribution Identification tool in Minitab, based on the p-value associated with the goodness-of-fit tests.

For each environment, the compatibility of the data with different statistical distributions was evaluated, and the distribution providing the best fit to the experimental data was selected. Descriptive statistical parameters, including mean and standard deviation, were also calculated to support the interpretation of data variability. Graphical tools, such as histograms and boxplots, were used to provide a visual assessment of data distribution and dispersion across protocol revisions.

4.1.1 Via Bove facility

The following tables summarize the descriptive statistical analyses performed on particulate, surface microbiological, and airborne microbiological data collected in the Bove facility.
Only the most representative environments were selected, based on the number of available observations and their relevance within the production process. In addition, some characteristic environments, such as the Airlock area, were included because of their functional role as transition zones between ISO 8 changing rooms and the ISO 7 cleanroom.

Particulate Analysis

Table 4.1 summarizes the descriptive statistics and the results of the Distribution Identification analysis performed on particulate monitoring data collected in the most representative environments of the Bove facility across the different revisions of the PL048-23 protocol. For each monitored environment, the number of observations, mean value, standard deviation, identified theoretical distribution, and corresponding goodness-of-fit p-value are reported.

The identified distributions were subsequently used as the basis for the statistical analyses presented in the following sections, including process capability analysis and inferential statistical tests.

Table 4.1 Mean values, standard deviations, identified distributions, and associated p-values for particulate measurements collected in the Bove facility.

ID	ROOM	ISO	PL048-23 Rev.	N	Mean	St.Dv.	DISTRIBUTION	P-VALUE
14	Clean Room	7	02	25	19828	23758	Johnson	0,984
			04	75	23552	27275	Johnson	0,86
			05	100	40789	41788	Johnson	0,992
13	Airlock	7	02	3	94888	38845	Johnson	0,719
			04	9	91369	58891		
			05	12	52112	33754		
23	Manufacturing cassette	7	02	10	21553	14105	Johnson	0,932
			04	30	22030	18295		
			05	40	33568	41661		
12	Spogliatoio donna	8	02	6	321737	145108	Box-Cox	0,139
			04	18	279731	201941		
			05	24	327281	249634		
11	Spogliatoio uomo	8	02	5	65559	17630	Box-Cox	0,905
			04	15	211116	159962	Johnson	0,617
			05	20	163980	170739	Johnson	0,812
15	Incoming Control room	7	02	6	31470	4742,3	Normal	0,458
			04	18	33565	16136		
			05	24	30532	19452		
21	Incoming Material room	7	02	6	4637,7	1411,4	Box-Cox Lognormal	0,821
			04	18	26757	20335	Box-Cox	0,946
			05	24	18432	14768	Johnson	0,857

The graphical analyses presented below refer to the most representative environments of the Bove facility across the different revisions of the PL048-23 protocol. The Clean Room environment is illustrated through both histogram and boxplot analyses, whereas the remaining monitored environments are presented by means of boxplots to facilitate the comparison of data distribution and variability among protocol revisions. Probability plots and goodness-of-fit tests are also reported to illustrate the distribution identification process and the selection of the theoretical model adopted for the subsequent statistical analyses.

The Clean Room environment was first analyzed, as it represents the most critical production area within the Bove facility. Figure 4.1 presents the histogram and boxplot of the transformed particulate data collected during Revisions 02, 04, and 05 of the PL048-23 protocol.

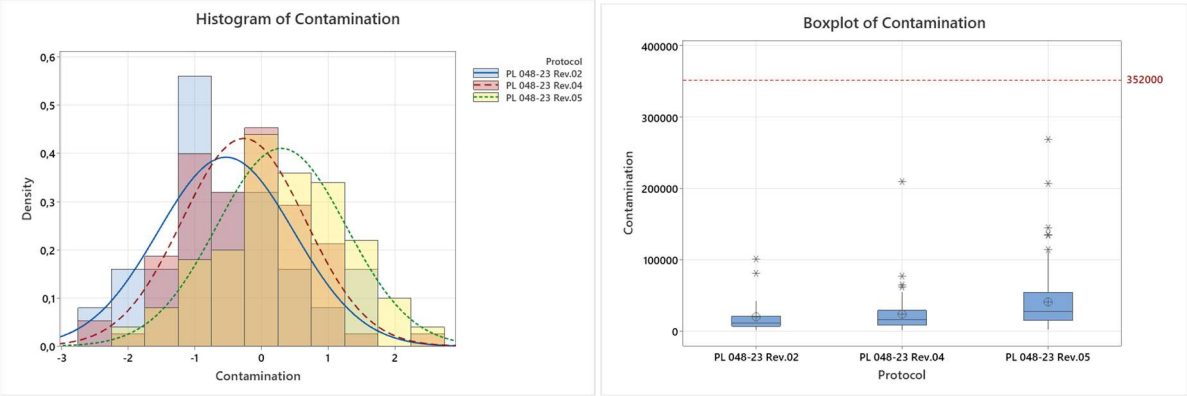
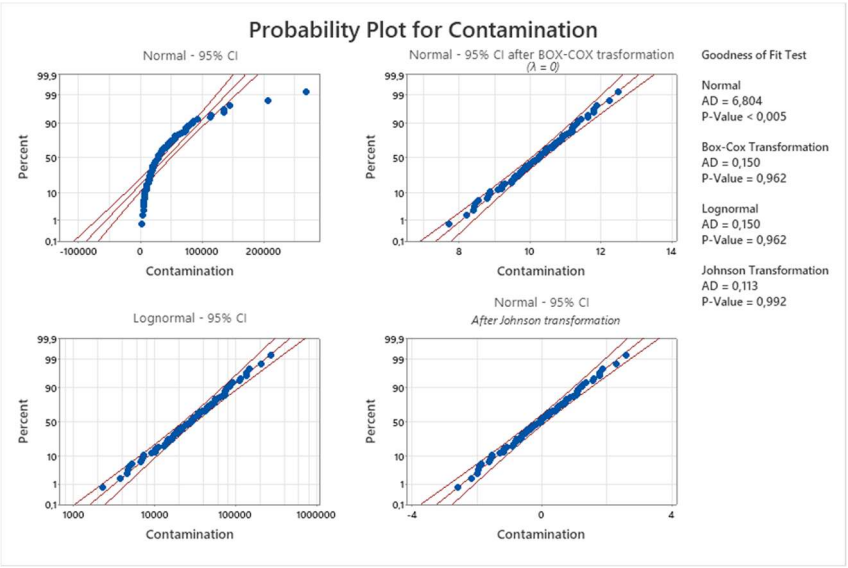


Figure 4.1 Histogram and boxplot of trasformed particulate data collected in the Clean Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

As shown in Figure 4.1, the transformed particulate datasets exhibited different distribution patterns across the three protocol revisions. Revision 02 was characterized by a distribution shifted toward lower contamination values, whereas Revision 05 showed a rightward shift, indicating higher particulate counts. This trend is also reflected in the boxplot, where Revision 05 exhibited a higher median and a wider interquartile range than the previous revisions, indicating increased variability. Despite this greater dispersion, all observations remained below the established acceptance limit.

Since the same theoretical distribution was identified for all three protocol revisions, only the probability plot corresponding to Revision 05 is reported as a representative example (Figure 4.2).



Goodness of Fit Test

Distribution	AD	P	LRT P
Normal	6,804	<0,005	
Box-Cox Transformation	0,150	0,962	
Lognormal	0,150	0,962	
3-Parameter Lognormal	0,129	*	0,785
Exponential	1,561	0,027	
2-Parameter Exponential	0,715	0,240	0,002
Weibull	0,925	0,019	
3-Parameter Weibull	0,553	0,161	0,003
Smallest Extreme Value	14,100	<0,010	
Largest Extreme Value	2,147	<0,010	
Gamma	0,725	0,071	
3-Parameter Gamma	0,524	*	0,054
Logistic	3,735	<0,005	
Loglogistic	0,175	>0,250	
3-Parameter Loglogistic	0,197	*	0,572
Johnson Transformation	0,113	0,992	

Figure 4.2 Probability plots and goodness-of-fit tests for particulate data collected in the Clean Room environment of the Bove facility for revision 05 of the PL048-23 protocol

The goodness-of-fit analysis confirmed that the Johnson distribution provided the best fit for the transformed particulate dataset, as indicated by the highest p-value (0.992) and the lowest Anderson-Darling statistic (AD = 0.113). Furthermore, the probability plot after Johnson transformation shows that the experimental observations closely follow the theoretical distribution, confirming the effectiveness of the applied transformation.

The same statistical approach was subsequently applied to the Airlock environment. Because only three observations were available for Revision 02, this dataset was excluded from the graphical distribution analysis. Consequently, only particulate monitoring data collected during Revisions 04 and 05 were considered.
Figure 4.3 presents the boxplot of transformed particulate data collected in the Airlock environment during Revisions 04 and 05 of the PL048-23 protocol.

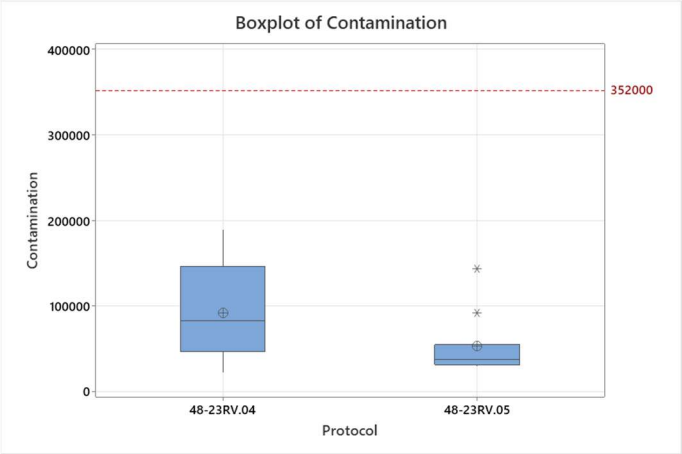
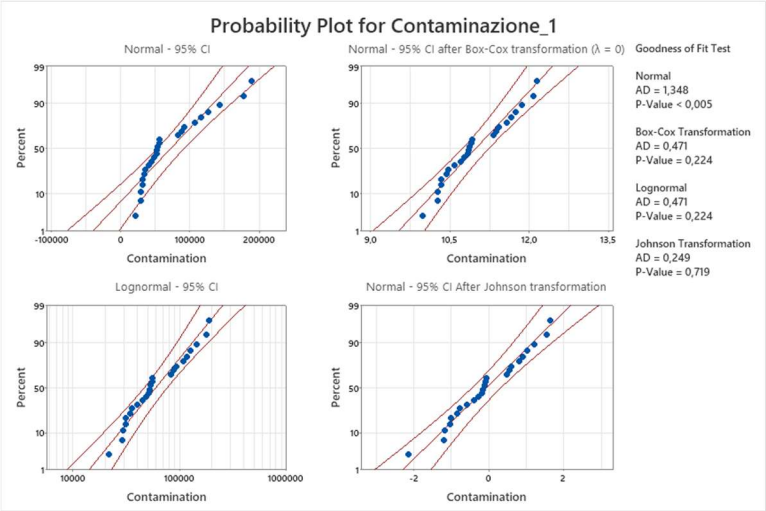


Figure 4.3 Boxplot for revisions 04 and 05 of the PL048-23 protocol for transformed particulate data collected in the Airlock environment of the Bove facility.

As shown in Figure 4.3, Revision 05 exhibited a narrower interquartile range and lower overall variability than Revision 04, indicating that most observations were concentrated within a smaller range of contamination values. Although a limited number of outliers was still present in Revision 05, the overall dispersion of the measurements was reduced compared with the previous revision.

The corresponding probability plots and goodness-of-fit tests are reported in Figure 4.4 to identify the theoretical distribution providing the best fit to the experimental data.



Goodness of Fit Test

Distribution	AD	P
Normal	1,348	<0,005
Box-Cox Transformation	0,471	0,224
Lognormal	0,471	0,224
Johnson Transformation	0,249	0,719

Figure 4.4 Probability plots and goodness-of-fit tests for particulate data collected in the Airlock environment of the Bove facility of the PL048-23 protocol.

The Distribution Identification analysis identified the Johnson distribution as the best-fitting model for the transformed Airlock dataset, as indicated by the lowest Anderson–Darling statistic (AD = 0.249) and the highest p-value (0.719). Although the Box-Cox transformation and the lognormal distribution also provided acceptable fits, the Johnson transformation showed the best overall agreement with the experimental data and was therefore selected for the subsequent statistical analyses..

The Manufacturing Cassette environment was subsequently analyzed using the same statistical approach. Figure 4.5 presents the boxplot of transformed particulate data collected during Revisions 02, 04, and 05 of the PL048-23 protocol.

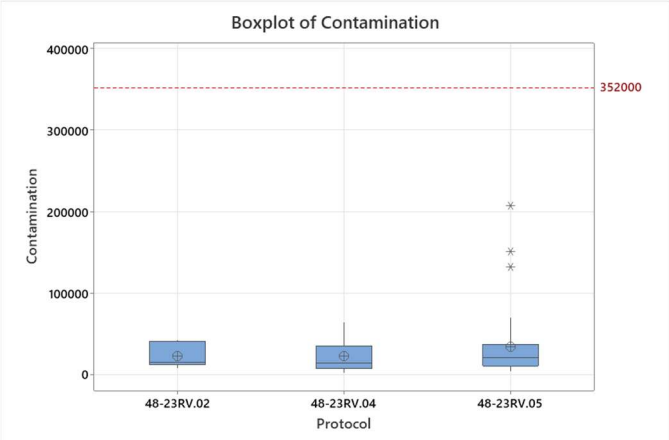
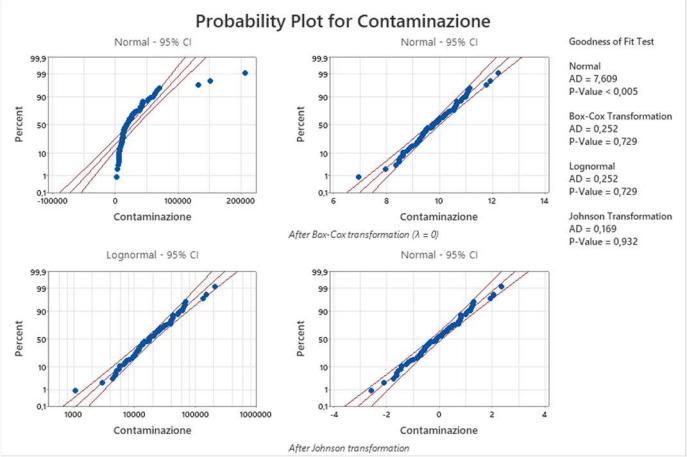


Figure 4.5 Boxplot of transformed particulate data collected in the Manufacturing cassette environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol

As shown in Figure 4.5, the three protocol revisions exhibited comparable median particulate contamination values, indicating a similar central tendency across the datasets. Revision 05 showed a slightly wider interquartile range and a greater number of high-value outliers than the previous revisions, reflecting increased data variability. Nevertheless, all observations remained below the established acceptance limit, confirming compliance with the required particulate contamination specifications.

The corresponding probability plots and goodness-of-fit tests are reported in Figure 4.6 to identify the theoretical distribution that best fitted the experimental data.



Goodness of Fit Test

Distribution	AD	P
Normal	7,609	<0,005
Box-Cox Transformation	0,252	0,729
Lognormal	0,252	0,729
Johnson Transformation	0,169	0,932

Figure 4.6 Probability plots and goodness-of-fit tests for particulate data collected in the Manufacturing Cassette environment of the Bove facility for the PL048-23 protocol

The Distribution Identification analysis confirmed the Johnson distribution as the best-fitting model for the Manufacturing Cassette dataset. The Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.169) and the highest p-value (0.932), confirming its suitability for the subsequent statistical analyses.

The same distribution and variability analyses were subsequently performed for the Women's and Men's Changing Room environments across Revisions 02, 04, and 05 of the PL048-23 protocol.
Figure 4.7 presents the boxplot of particulate contamination measured in the Women's Changing Room environment during Revisions 02, 04, and 05 of the PL048-23 protocol.

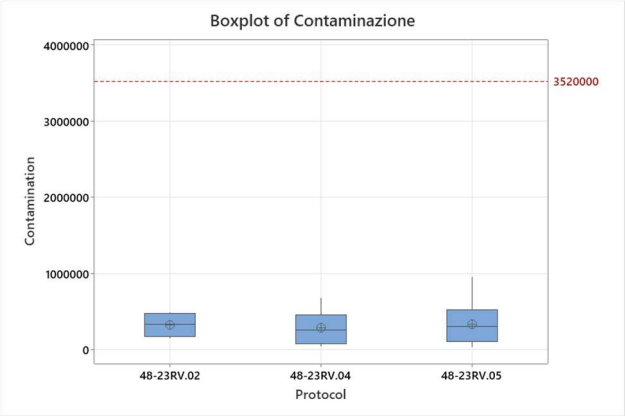
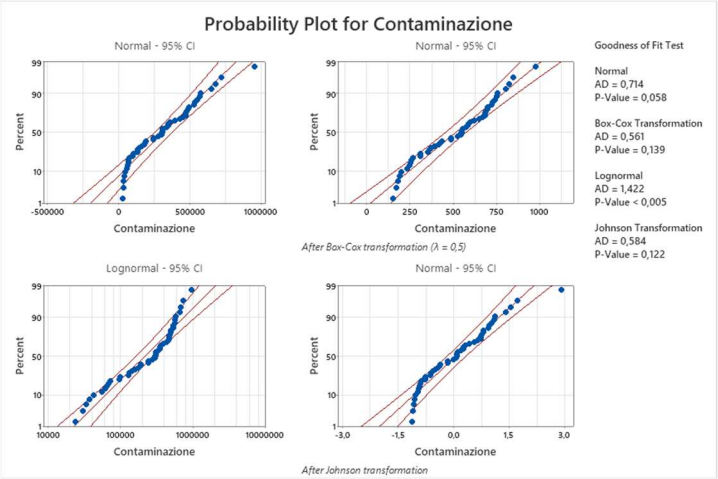


Figure 4.7 Boxplot of particulate data collected in the Women's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol

The three protocol revisions exhibited comparable distributions, with similar median values and interquartile ranges. Although Revision 05 showed a slightly wider range of observations, no substantial differences in data variability were observed among the investigated revisions. All measured values remained below the established acceptance limit, confirming compliance with the required particulate contamination specifications.

The corresponding probability plots and goodness-of-fit tests are reported in Figure 4.8.



Goodness of Fit Test

Distribution	AD	P
Normal	0,714	0,058
Box-Cox Transformation	0,561	0,139
Lognormal	1,422	<0,005
Johnson Transformation	0,584	0,122

Figure 4.8 Probability plots and goodness-of-fit tests for particulate data collected in the Women's Changing Room environment of the Bove facility for the PL048-23 protocol.

The Box-Cox transformation provided the best overall fit, as indicated by the lowest Anderson–Darling statistic (AD = 0.561) and the highest p-value (0.139). Although the Johnson transformation also showed satisfactory agreement with the experimental data, the lognormal distribution exhibited the poorest goodness-of-fit.

Figure 4.9 presents the boxplot of particulate contamination measured in the Men's Changing Room environment during Revisions 02, 04, and 05 of the PL048-23 protocol.

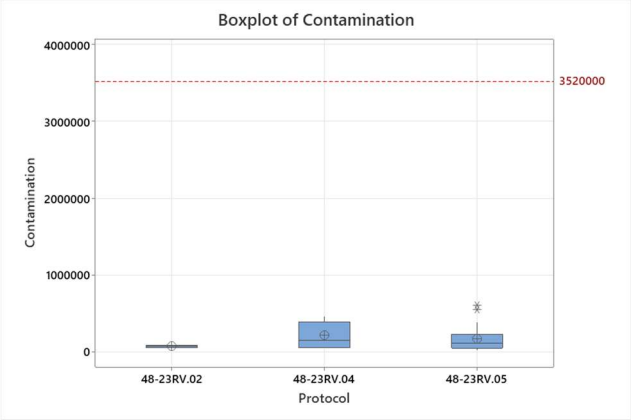
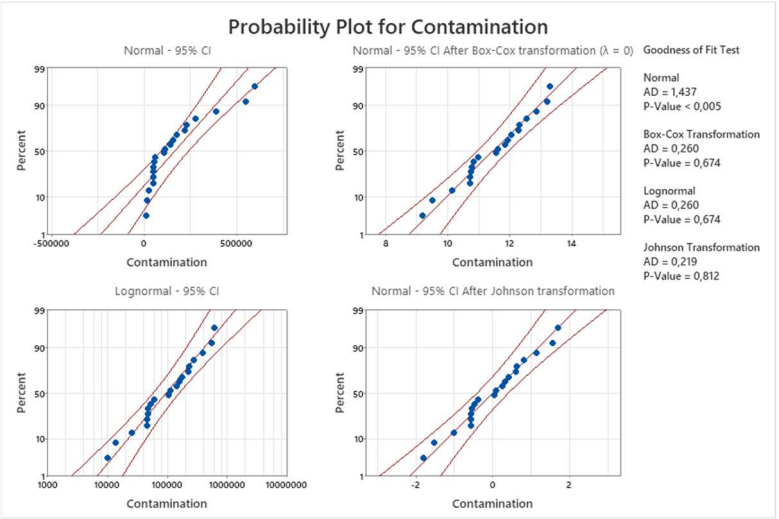


Figure 4.9 Boxplot of data collected in the Men's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Compared with Revision 02, Revisions 04 and 05 exhibited greater data variability, with Revision 04 showing the widest interquartile range. Although several high-value outliers were observed in Revision 05, all measurements remained below the established acceptance limit, indicating compliance with the required particulate contamination specifications.

Since the same theoretical distribution was identified for all three protocol revisions, only the probability plot corresponding to Revision 05 is reported as a representative example (Figure 4.10).



Goodness of Fit Test

Distribution	AD	P
Normal	1,437	<0,005
Box-Cox Transformation	0,260	0,674
Lognormal	0,260	0,674
Johnson Transformation	0,219	0,812

Figure 4.10 Probability plots and goodness-of-fit tests for particulate data collected in the Men's Changing Room environment of the Bove facility for revision 05 of the PL048-23 protocol.

The Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.219) and the highest p-value (0.812), indicating the best agreement between the experimental data and the theoretical distribution. Although the Box-Cox transformation and the lognormal distribution also provided acceptable fits, the Johnson transformation was selected for the subsequent statistical analyses.

Finally, the same statistical approach was applied to the Incoming Control Room and Incoming Material Room environments, completing the graphical evaluation of the most representative monitored areas within the Bove facility.
Figure 4.11 showed the Box plot of Incoming Control room.

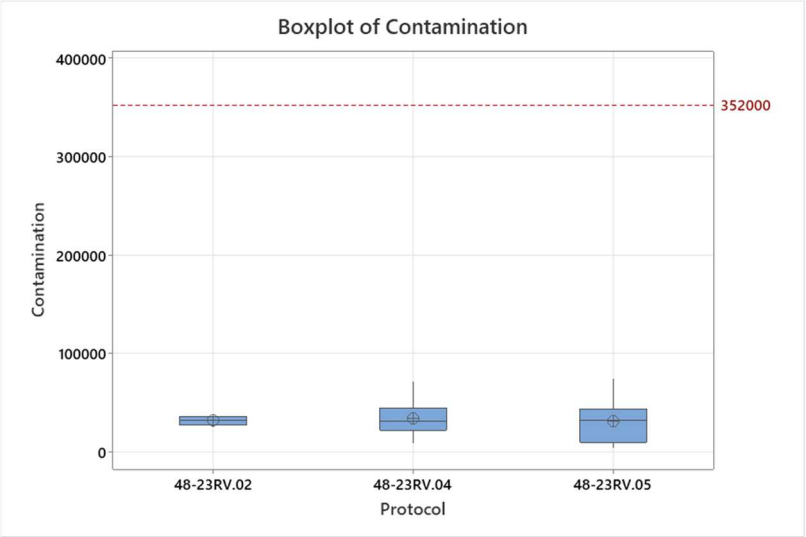
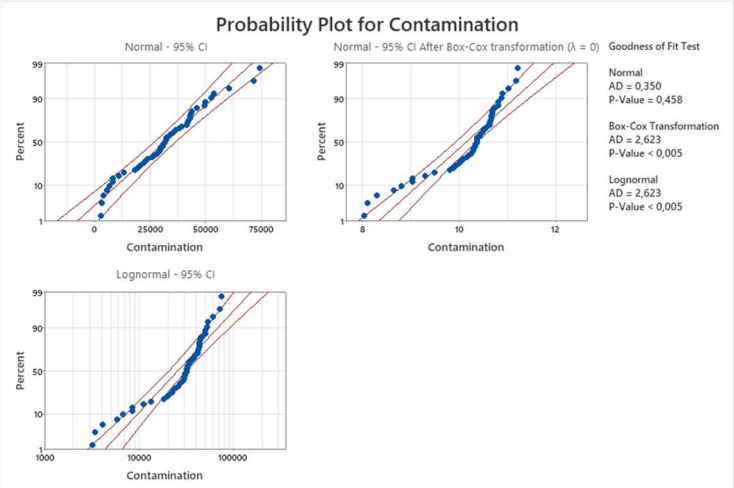


Figure 4.11 Boxplot of transformed particulate data collected in the Incoming Control Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

The three protocol revisions exhibited comparable median contamination values and similar levels of variability. Although Revision 05 showed a slightly wider interquartile range than the previous revisions, no substantial differences in data dispersion were observed. All recorded particulate contamination values remained below the established acceptance limit throughout the investigated monitoring periods.

The corresponding probability plots and goodness-of-fit tests are presented in Figure 4.12



Goodness of Fit Test

Distribution	AD	P
Normal	0,350	0,458
Box-Cox Transformation	2,623	<0,005
Lognormal	2,623	<0,005

Figure 4.12 Probability plots and goodness-of-fit tests for particulate data collected in the Incoming Control Room environment of the Bove facility for the PL048-23 protocol.

As shown in Figure 4.12, the normal distribution provided the best fit to the experimental data, yielding the lowest Anderson–Darling statistic (AD = 0.350) and the highest p-value (0.458). In contrast, both the Box-Cox transformation and the lognormal distribution showed poor goodness-of-fit, with p-values below 0.005. Consequently, no data transformation was required for the subsequent statistical analyses.

Figure 4.13 presents the boxplot of particulate contamination measured in the Incoming Material Room during Revisions 02, 04, and 05 of the PL048-23 protocol.

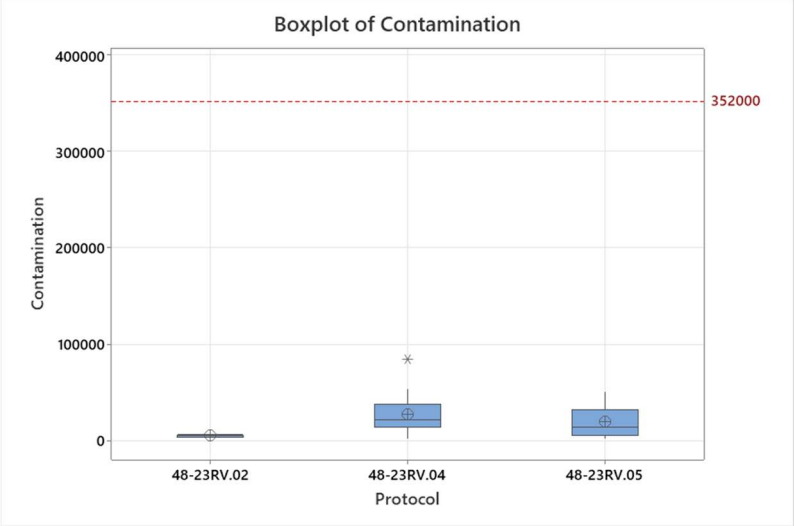
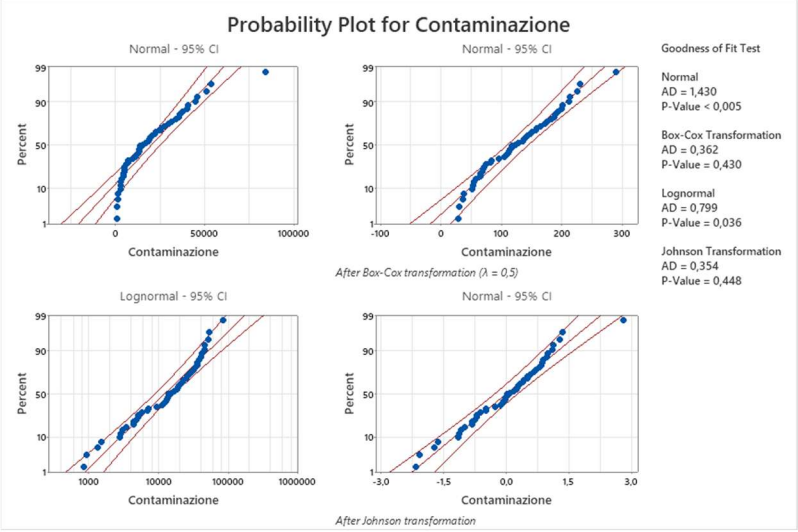


Figure 4.13 Boxplot of transformed particulate data collected in the Incoming Material Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 02 exhibited the lowest variability, whereas Revisions 04 and 05 showed wider interquartile ranges and greater overall data dispersion. A single high-value outlier was observed in Revision 04, while the overall distribution of Revision 05 remained comparable to that of Revision 04. Nevertheless, all measurements remained below the established acceptance limit throughout the investigated monitoring periods.

Since the same theoretical distribution was identified for all protocol revisions, only the probability plot corresponding to Revision 05 is reported as a representative example (Figure 4.14).



Goodness of Fit Test

Distribution	AD	P
Normal	1,430	<0,005
Box-Cox Transformation	0,362	0,430
Lognormal	0,799	0,036
Johnson Transformation	0,354	0,448

Figure 4.14 Probability plots and goodness-of-fit tests for particulate data collected in the Incoming Material Room environment of the Bove facility for revision 05 of the PL048-23 protocol

The Johnson transformation provided the best overall fit, yielding the lowest Anderson–Darling statistic (AD = 0.354) and the highest p-value (0.448). Although the Box-Cox transformation produced very similar results (AD = 0.362; p = 0.430), the Johnson transformation was selected for the subsequent statistical analyses.

In addition to particulate monitoring, surface microbiological analyses were performed in the selected environments of the Bove facility to evaluate microbiological contamination trends across the different revisions of the PL048-23 protocol. Consistent with the statistical approach described in Section 3.4, microbiological datasets were evaluated using descriptive graphical methods only.

Surface Microbiological Analysis

Table 4.2 summarizes the descriptive statistics of the surface microbiological measurements collected in the most representative environments of the Bove facility across the different revisions of the PL048-23 protocol. For each monitored environment, the number of observations, mean value, and standard deviation are reported.

Table 4.2 Descriptive statistical analysis of surface microbiological measurements collected in the monitored environments of the Bove facility.

ID	ROOM	ISO	PL048-23 Rev.	N	Mean	St.Dv.
14	Clean Room	7	02	11	6,1818	9,1632
			04	35	2,2571	3,9656
			05	80	3,225	4,8860
13	Airlock	7	02	4	17	11,916
			04	12	1,9167	5,4016
			05	16	0,625	0,88506
23	Manufacturing cassette	7	02	7	0,86	1,07
			04	21	1,57	2,56
			05	40	1,825	2,7446
12	Spogliatoio donna	8	02	5	7,8	9,3648
			04	15	7,7333	12,809
			05	20	8,4	17,080
11	Spogliatoio uomo	8	02	5	3,6	3,5071
			04	15	8,2	12,571
			05	20	1,45	2,5231
15	Incoming Control room	7	02	5	1,2	1,7889
			04	15	0,93	1,2228
			05	20	0,25	0,63867
21	Incoming Material room	7	02	5	1,6	3,5777
			04	15	0,4	0,63246
			05	24	1,4583	2,1260

The graphical analyses presented below refer to the most representative environments of the Bove facility across Revisions 02, 04, and 05 of the PL048-23 protocol. The Clean Room environment is illustrated through both histogram and boxplot analyses, whereas the remaining monitored environments are presented by means of boxplots to facilitate the comparison of microbiological contamination levels and data variability among protocol revisions.

The Clean Room environment was first analyzed, as it represents the most critical production area within the Bove facility. Figure 4.15 presents the histograms of surface microbiological contamination collected during Revisions 02, 04, and 05 of the PL048-23 protocol.

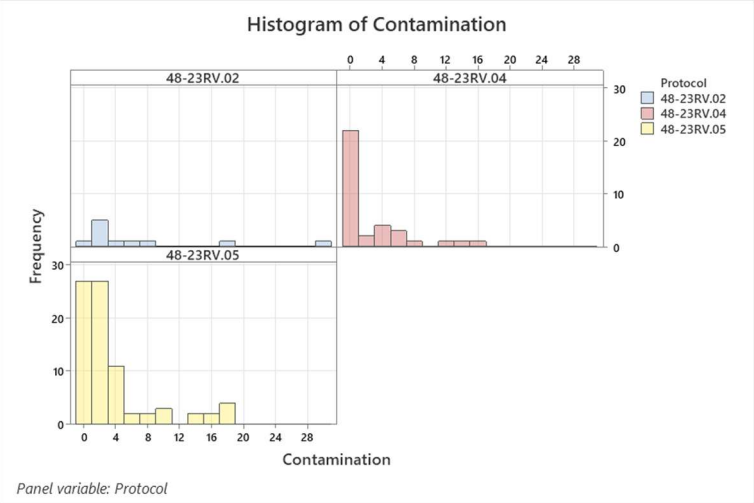


Figure 4.15 Histogram of surface microbiological contamination collected in the Clean Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

As shown in Figure 4.15, the majority of the observations were concentrated at low contamination levels throughout the three protocol revisions, whereas only a limited number of measurements exhibited higher colony counts. This distribution is consistent with the low microbiological contamination generally expected in classified cleanroom environments.

Since the acceptance limits differ between floor samples and the remaining monitored surfaces, the corresponding boxplots are presented separately in Figure 4.16.

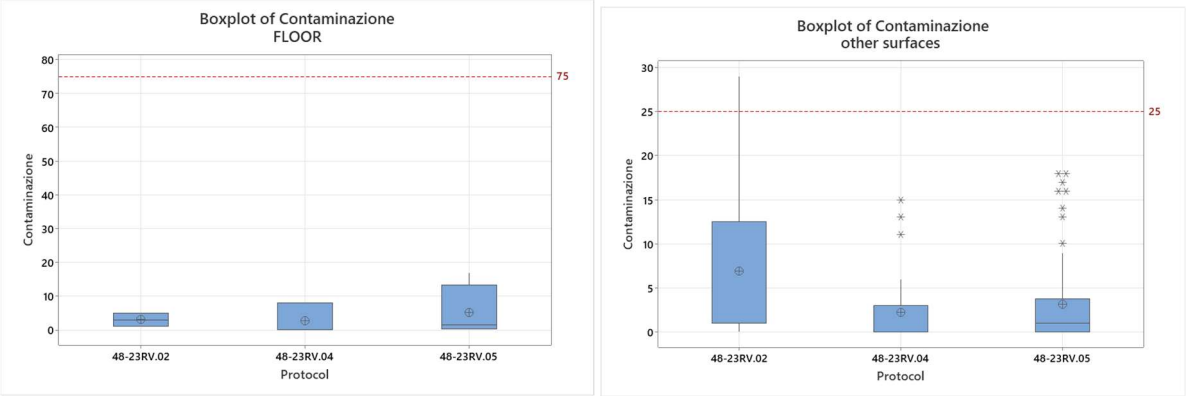


Figure 4.16 Boxplot representation of surface microbiological contamination measured on floor surfaces and other monitored positions in the Clean Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Figure 4.16 presents the boxplots of surface microbiological contamination measured on floor surfaces and on the remaining monitored positions in the Clean Room environment. Floor samples showed consistently low contamination levels throughout the three protocol revisions, although Revision 05 exhibited a slight increase in both the median value and data dispersion. A similar trend was observed for the remaining monitored surfaces, where most observations remained concentrated at low contamination levels despite the presence of a limited number of isolated outliers. In both sampling categories, all recorded values complied with the corresponding acceptance limits.

The same descriptive graphical approach was subsequently applied to the Airlock environment. The results are summarized in the boxplots shown in Figure 4.17.

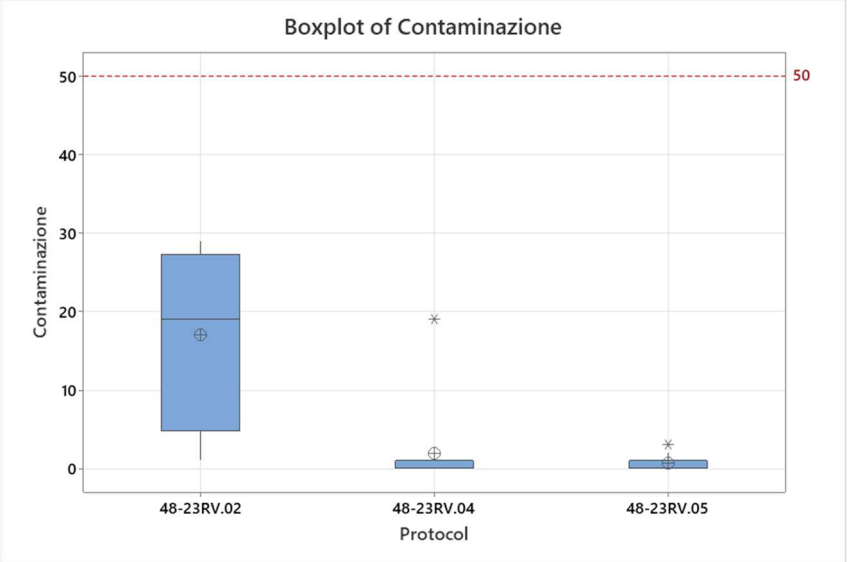


Figure 4.17 Boxplot representation of surface microbiological contamination measured in the Airlock environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Figure 4.17 shows the boxplot of surface microbiological contamination measured in the Air Lock environment for Revisions 02, 04, and 05 of the PL048-23 protocol. Compared with Revision 02, Revisions 04 and 05 exhibited substantially lower contamination levels and a marked reduction in data dispersion, with most observations concentrated around zero CFU. Only a limited number of isolated outliers were observed in the two most recent revisions. Overall, all recorded values remained below the established acceptance limit throughout the investigated protocol revisions.

The same descriptive graphical approach was subsequently applied to the Manufacturing Cassette environment. Figure 4.18 presents the boxplots of surface microbiological contamination measured on floor surfaces and on the remaining monitored positions during Revisions 02, 04, and 05 of the PL048-23 protocol.

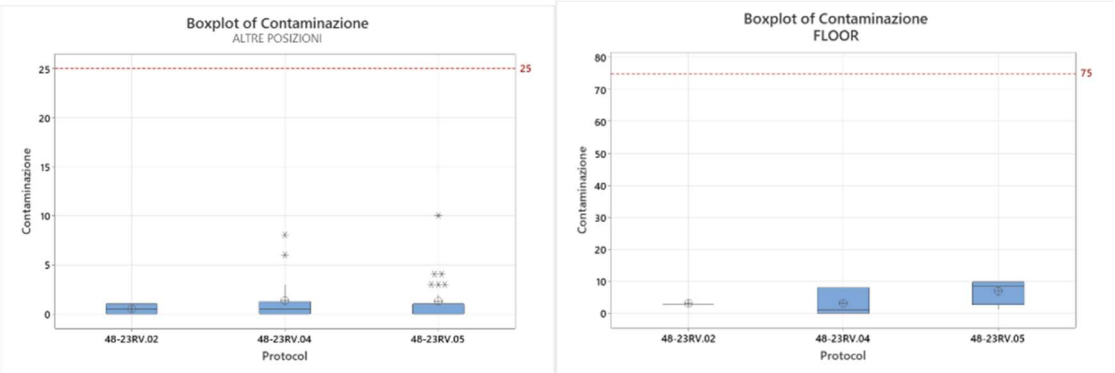


Figure 4.18 Boxplot representation of surface microbiological contamination measured in the Manufacturing Cassette environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

For the floor samples, a slight increase in both the median contamination values and data dispersion was observed from Revision 02 to Revision 05. In contrast, the remaining monitored positions exhibited consistently low contamination levels throughout the investigated protocol revisions, although a limited number of isolated outliers was observed in Revisions 04 and 05. In both sampling categories, all recorded values complied with the corresponding acceptance limits.

The same descriptive graphical analyses were subsequently performed for the Men's and Women's Changing Room environments across Revisions 02, 04, and 05 of the PL048-23 protocol.
Figure 4.19 presents the boxplots of surface microbiological contamination measured on floor surfaces and on the remaining monitored positions in the Men's Changing Room environment during Revisions 02, 04, and 05 of the PL048-23 protocol.

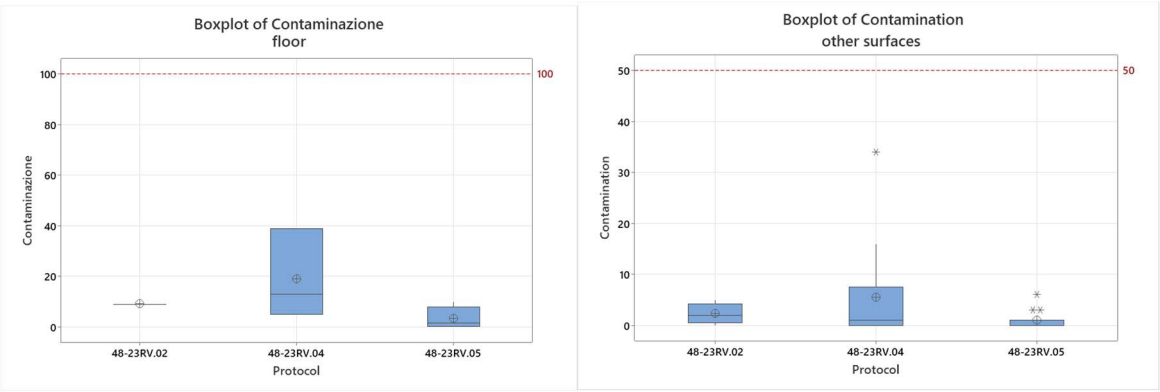


Figure 4.19 Boxplot representation of surface microbiological contamination measured in the Men's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol

Floor samples exhibited the highest variability during Revision 04, whereas lower contamination levels and reduced data dispersion were observed in Revision 05. A similar trend was identified for the remaining monitored positions, where Revision 04 showed greater variability together with a limited number of high-value outliers, while Revision 05 was characterized by measurements predominantly concentrated close to zero. In both sampling categories, all recorded values complied with the corresponding acceptance limits.

Figure 4.20 presents the boxplots of surface microbiological contamination measured on floor surfaces and on the remaining monitored positions in the Women's Changing Room environment during Revisions 02, 04, and 05 of the PL048-23 protocol.

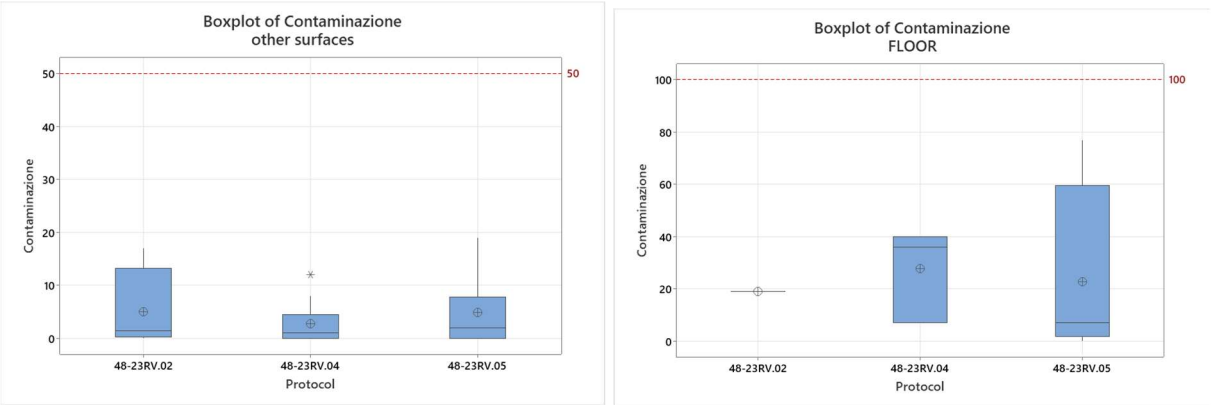


Figure 4.20 Boxplot representation of surface microbiological contamination measured in the Women's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

The remaining monitored positions exhibited consistently low contamination levels throughout the investigated protocol revisions, although a slight increase in data dispersion was observed in Revision 05. Floor samples showed greater variability during Revisions 04 and 05 than in Revision 02, with the widest distribution of observations recorded in the most recent revision. Nevertheless, all measurements complied with the corresponding acceptance limits.

Finally, the same descriptive graphical analyses were applied to the Incoming Control Room and Incoming Material Room environments, completing the evaluation of surface microbiological contamination in the most representative monitored areas of the Bove facility. Figure 4.21 shows the boxplots of surface microbiological contamination measured on floor surfaces and other monitored positions in the Incoming Control Room environment for Revisions 02, 04, and 05 of the PL048-23 protocol.

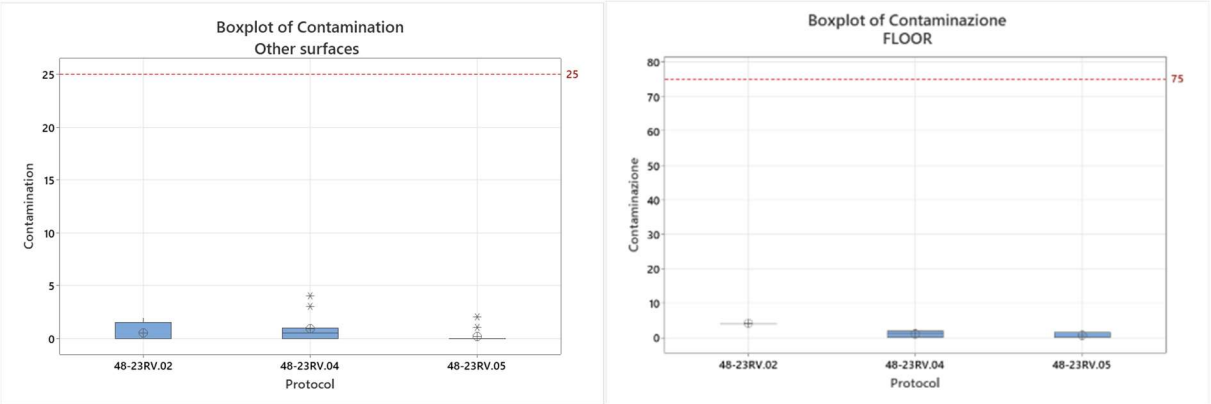


Figure 4.21 Boxplot representation of surface microbiological contamination measured in the Incoming Control Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Overall, low contamination levels were observed throughout the investigated monitoring periods. Floor samples showed only minor differences in median values and data dispersion, whereas the remaining monitored positions exhibited measurements predominantly concentrated around zero, with only a few isolated outliers in Revisions 04 and 05. All recorded values complied with the corresponding acceptance limits.

Figure 4.22 presents the boxplots of surface microbiological contamination measured on floor surfaces and on the remaining monitored positions in the Incoming Material Room environment during Revisions 02, 04, and 05 of the PL048-23 protocol.

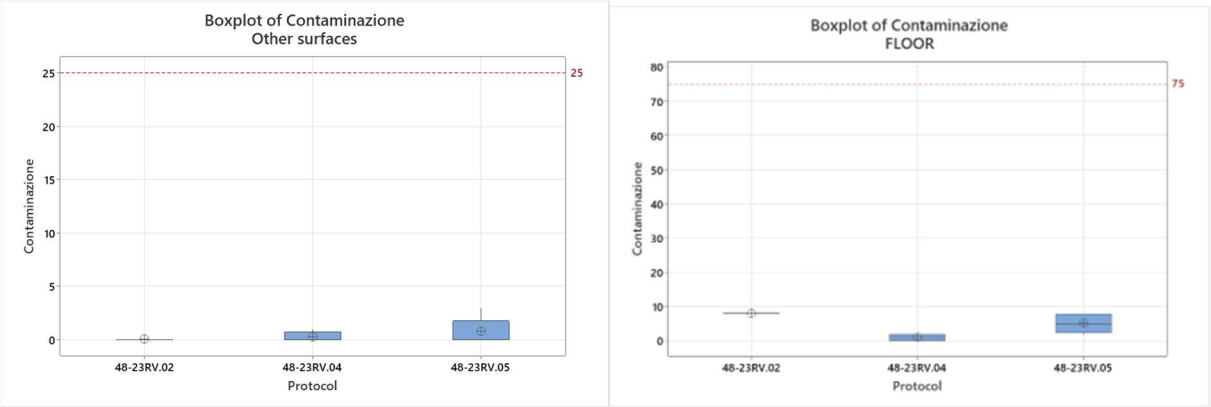


Figure 4.22 Boxplot representation of surface microbiological contamination measured in the Incoming Material Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol

Floor samples exhibited a slight increase in both median contamination values and data dispersion during Revision 05 compared with the previous revisions. A similar trend was observed for the remaining monitored positions, although contamination levels remained generally low and only limited variability was detected. In both sampling categories, all measured values complied with the corresponding acceptance limits.

Finally the airborne microbiological analyses were also performed in the selected environments of the Bove facility across Revisions 02, 04, and 05 of the PL048-23 protocol. Consistent with the statistical approach described in Section 3.4, airborne microbiological datasets were evaluated using descriptive graphical methods only.

Airborne Microbiological Analysis

Table 4.3 summarizes the descriptive statistics of the airborne microbiological measurements collected in the most representative environments of the Bove facility. For each monitored environment, the number of observations, mean value, and standard deviation are reported.

Table 4.3 Mean values and standard deviations associated with airborne microbiological measurements collected in the monitored environments.

ID	ROOM	ISO	PL048-23 Rev.	N	Mean	St.Dv.
14	Clean Room	7	02	10	29	26,854
			04	30	6,6667	9,5893
			05	40	17,85	23,515
			02	1	10	/

13	Airlock	7	04	3	33,333	40,415
			05	4	20	27,080
23	Manufacturing cassette	7	02	4	25	17,321
			04	12	30,833	28,749
			05	16	20	23,094
12	Spogliatoio donna	8	02	3	83,333	42,633
			04	9	75,556	49,272
			05	12	48,333	52,020
11	Spogliatoio uomo	8	02	2	20	14,142
			04	6	70	31,623
			05	8	16,25	14,079
15	Incoming Control room	7	02	2	30	14,142
			04	6	23,333	20,656
			05	8	17,5	18,323
21	Incoming Material room	7	02	2	30	14,142
			04	6	18,333	27,869
			05	8	7,5	10,351

The Clean Room environment is illustrated through both histogram and boxplot analyses, whereas the remaining monitored environments are presented by means of boxplots to facilitate the comparison of airborne microbiological contamination levels and data variability among protocol revisions.

Airborne microbiological measurements collected in the Clean Room environment were analyzed for revisions 02, 04, and 05 of the PL048-23 protocol showed in Figure 4.23.

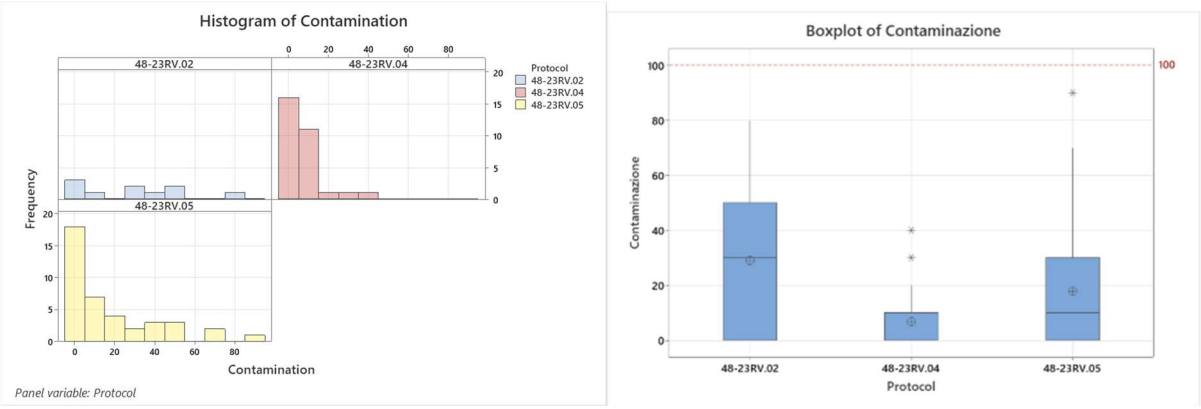


Figure 4.23 Histogram and Boxplot representation of airborne microbiological contamination measured in the Clean Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

As shown in Figure 4.23, most observations were concentrated at low contamination levels throughout the three protocol revisions, although a limited number of higher colony counts was observed. The boxplot highlights differences among the three datasets, with Revision 02 showing the highest median contamination level, whereas Revision 04 exhibited the lowest median and the lowest data dispersion. Revision 05 showed intermediate contamination levels together with a wider distribution of observations and a limited number of isolated high-value measurements. Nevertheless, all recorded values complied with the established acceptance limit.

The same descriptive graphical approach was subsequently applied to the Airlock environment. Figure 4.24 presents the boxplot of airborne microbiological contamination measured during Revisions 02, 04, and 05 of the PL048-23 protocol.

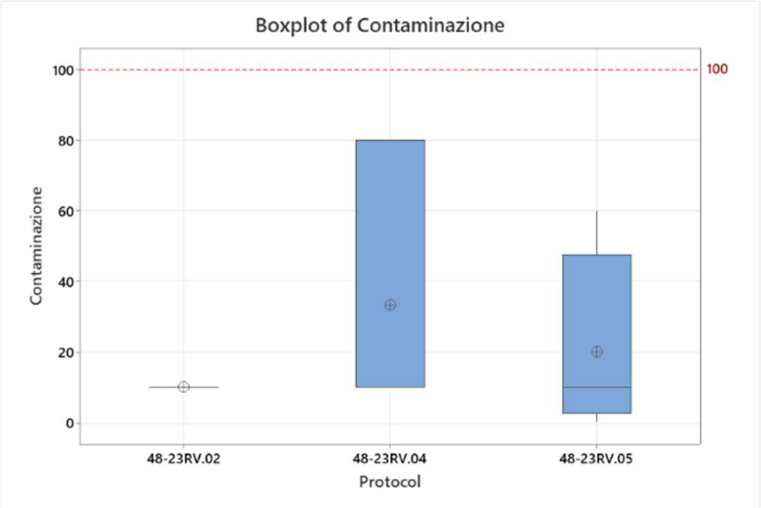


Figure 4.24 Boxplot representation of airborne microbiological contamination measured in the Airlock environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol

Because only a single observation was available for Revision 02, the corresponding distribution could not be evaluated. Compared with Revision 05, Revision 04 exhibited higher median contamination values and greater data variability, whereas Revision 05 showed lower median values together with a wider distribution of observations. Nevertheless, all recorded airborne microbiological contamination values complied with the established acceptance limit throughout the investigated protocol revisions.

Comparable analyses were also carried out for the Manufacturing Cassette environment across revisions 02, 04, and 05 of the PL048-23 protocol showed in Figure 4.25.

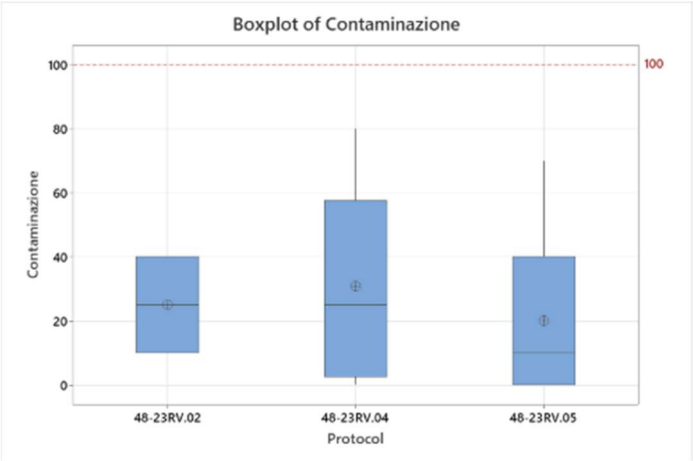


Figure 4.25 Boxplot representation of airborne microbiological contamination measured in the Manufacturing Cassette environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

The three protocol revisions exhibited comparable median contamination values, although Revision 04 showed the greatest data variability. Revision 05 was characterized by a lower median contamination level and a wider distribution of observations than Revision 02. Overall, all measured values complied with the established acceptance limit.

The same descriptive graphical analyses were subsequently performed for the Women's and Men's Changing Room environments across Revisions 02, 04, and 05 of the PL048-23 protocol showed in Figure 4.26 and Figure 4.27.

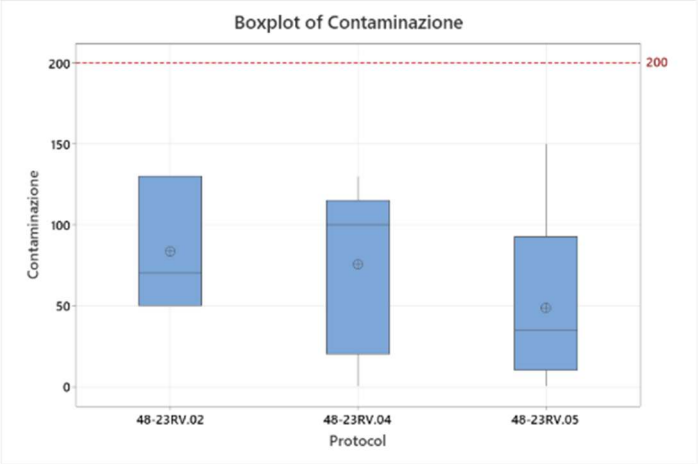


Figure 4.26 Boxplot representation of airborne microbiological contamination measured in the Women's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

The three protocol revisions exhibited comparable distributions, with Revision 05 showing lower median contamination values than Revisions 02 and 04. Although some variability was observed among the investigated revisions, all recorded measurements complied with the corresponding acceptance limit.

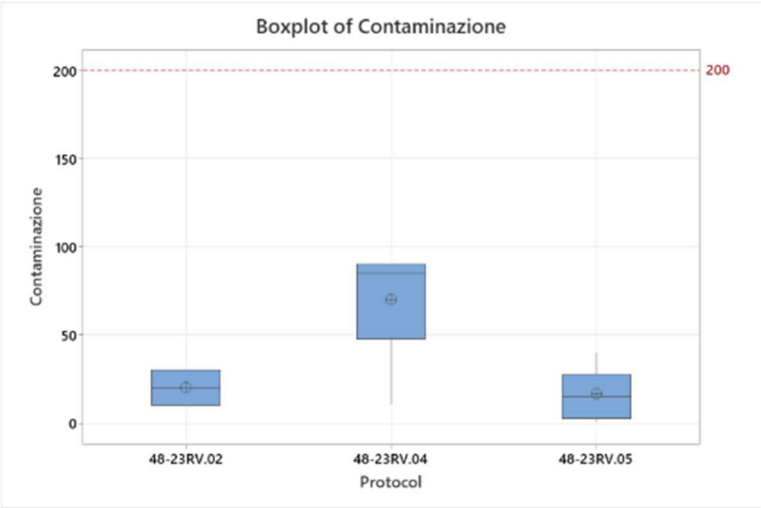


Figure 4.27 Boxplot representation of airborne microbiological contamination measured in the Men's Changing Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 04 exhibited the highest median contamination level together with the greatest data variability among the investigated protocol revisions. In contrast, Revisions 02 and 05 showed lower median contamination values and a narrower distribution of observations. Nevertheless, all recorded measurements complied with the established acceptance limit.

Finally, the same graphical analyses were extended to the Incoming Control Room and Incoming Material Room environments, completing the evaluation of airborne microbiological data for the most representative monitored areas of the Bove facility.
The Boxplots are showed in Figure 4.28 e 4.29.

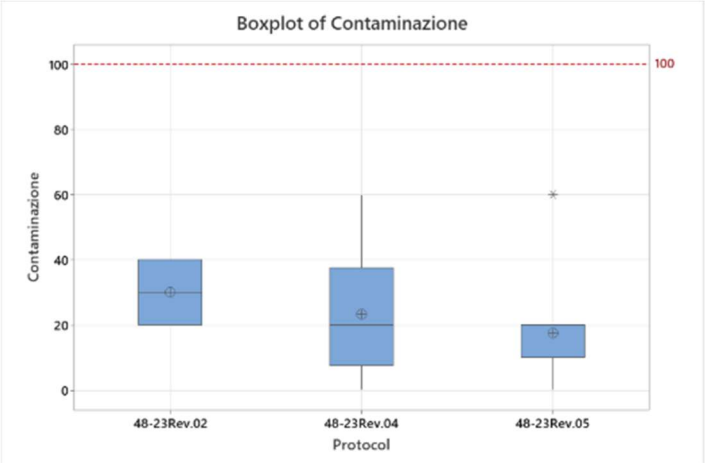


Figure 4.28 Boxplot representation of airborne microbiological contamination measured in the Incoming Control Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 02 exhibited the highest median contamination level, whereas Revisions 04 and 05 showed lower median values and reduced overall contamination. Although an isolated high-value observation was recorded in Revision 05, most measurements remained concentrated within a relatively narrow range. Overall, all recorded values complied with the established acceptance limit.

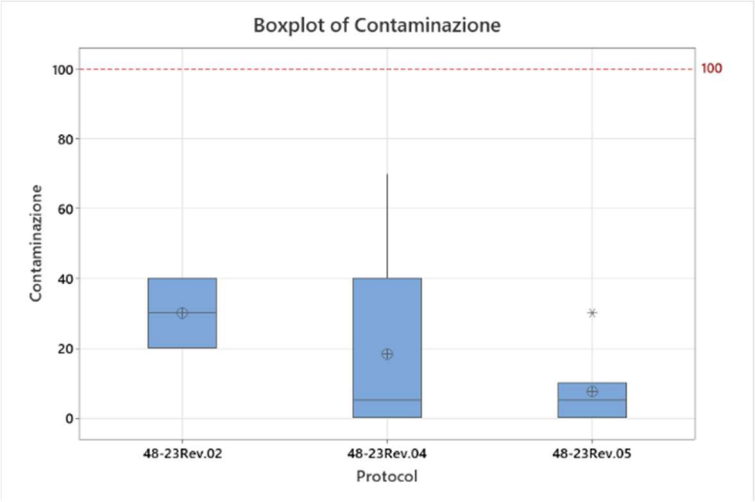


Figure 4.29 Boxplot representation of airborne microbiological contamination measured in the Incoming Material Room environment of the Bove facility for revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 02 showed the highest median contamination value among the investigated protocol revisions, whereas Revisions 04 and 05 exhibited lower median values. Revision 04 presented the greatest variability, while Revision 05 was characterized by contamination values predominantly concentrated at lower levels despite the presence of a single isolated outlier. All recorded measurements complied with the established acceptance limit.

The results presented in this section describe the statistical characteristics of particulate and microbiological environmental monitoring data collected in the Bove facility. The same analytical methodology was subsequently applied to the Via Statale facility, allowing the environmental monitoring performance of the two production sites to be evaluated using a consistent statistical approach.

4.1.2 Via Statale facility

The following tables summarize the descriptive statistical analyses performed on particulate, surface microbiological, and airborne microbiological monitoring data collected in the Via Statale facility. As for the Bove facility, only the most representative environments were selected according to the number of available observations and their relevance within the production process, including both production and transition areas characterized by different ISO classifications.

Particulate Analysis

The same statistical methodology described for the Bove facility was subsequently applied to the environmental monitoring data collected at the Statale facility. As for the previous analysis, particulate monitoring data were initially evaluated through descriptive statistics and Distribution Identification analysis in order to characterize the datasets and identify the theoretical distribution that best fitted each monitored environment and protocol revision. The main statistical parameters obtained for the particulate datasets, including the number of observations, mean value, standard deviation, identified distribution, and corresponding p-value, are summarized in Table 4.4.

Table 4.4 Mean values, standard deviations, identified distributions, and associated p-values for particulate measurements collected in the Statale facility.

ID	ROOM	ISO	PL0158Rev.	N	Mean	St.Dv.	DISTRIBUTION	P-VALUE
1	Clean room	7	05	88	39039	26733	Johnson	0,860
		8	02	42	59983	21470	Johnson	0,926
			03	241	150358	180207	Johnson	0,199
			04	44	131222	101745	Johnson	0,931
			05	22	34789	26916	Johnson	0,963
2	Inlet Materials room	8	02	10	122385	40298	Box-Cox	0,883
			03	55	468629	745468	Johnson	0,507
			04	10	182899	30170	Box-Cox	0,560
			05	25	82086	44348	Johnson	0,624
3	Outlet Materials room	8	02	10	175922	51785	Lognormal	0,307
			03	55	280930	183662	Johnson	0,934
			04	10	195494	56403	Lognormal	0,575
			05	25	109280	34228	Box-Cox	0,6
7	Airlock	8	02	12	301271	132285	Johnson	0,858
			03	72	698666	778385	Johnson	0,118
			04	12	1120540	320772	Johnson	0,321
			05	30	863345	240222	Johnson	0,869
5	Airlock Dressing room	8	05	30	802232	820931	Johnson	0,349
4	Airlock Washing room	8	05	30	434722	524842	Johnson	0,135

The graphical analyses presented below illustrate the distribution and variability of the particulate monitoring data collected in the selected environments.The analysis begins with the Clean Room environment, considering the ISO 7 area for Revision 05 and the ISO 8 area for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

The analysis begins with the Clean Room environment. Since this area was reclassified from ISO 8 to ISO 7 in Revision 05 of the PL0158 protocol, the particulate monitoring data are presented separately according to the corresponding classification.

Figure 4.30 presents the histogram of transformed particulate contamination measured in the Clean Room environment during Revision 05 (ISO 7).

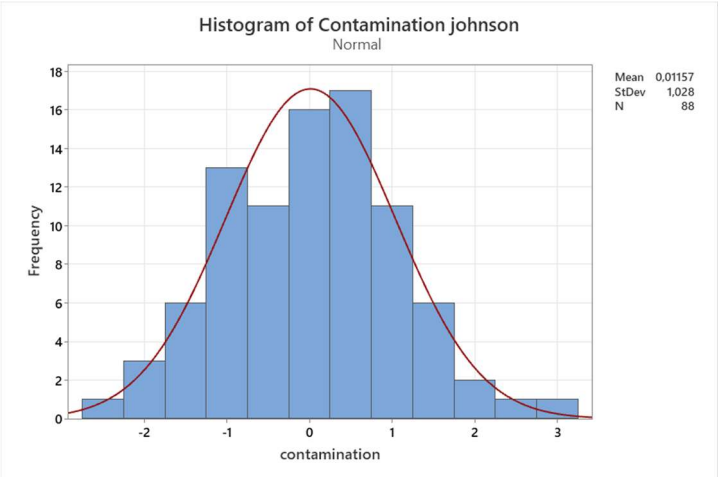
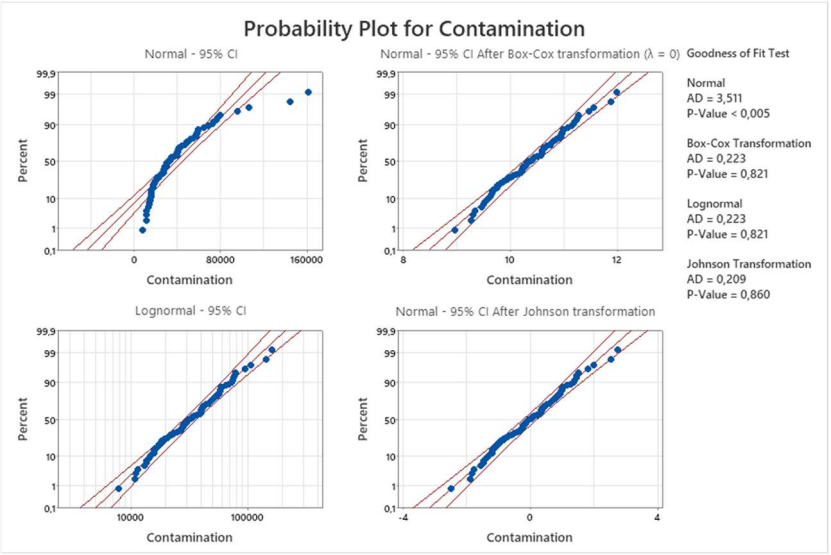


Figure 4.30 Histogram of trasfromed particulate contamination collected in the ISO 7 Clean Room environment of the Via Statale facility for revision 05 of the PL0158-23 protocol

Following the Johnson transformation, the particulate data exhibited an approximately symmetric distribution, showing good agreement with the fitted normal curve.

The corresponding probability plots and goodness-of-fit tests are reported in Figure 4.31



Goodness of Fit Test

Distribution	AD	P
Normal	3,511	<0,005
Box-Cox Transformation	0,223	0,821
Lognormal	0,223	0,821
Johnson Transformation	0,209	0,860

Figure 4.31 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the ISO 7 Clean Room environment of the Via Statale facility for revision 05 of the PL0158 protocol.

As shown in Figure 4.31, the Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.209) and the highest p-value (0.860), indicating the best agreement between the transformed data and the theoretical normal distribution. Similar results were also obtained with the Box-Cox transformation and the lognormal distribution; however, the Johnson transformation was selected for the subsequent statistical analyses because it provided the best overall goodness-of-fit.

The same environment was then evaluated considering the particulate monitoring data collected when it was classified as ISO 8 during Revisions 02, 03, 04, and 05.

Figure 4.32 shows the histograms of transformed particulate contamination measured in the ISO 8 Clean Room environment for Revisions 02, 03, 04, and 05 of the PL0158 protocol

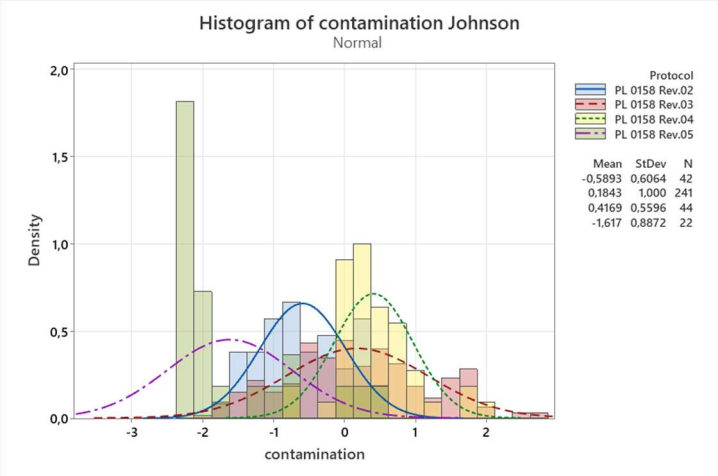


Figure 4.32 Histogram of trasfromed particulate contamination collected in the ISO 8 Clean Room environment of the Via Statale facility for revision 02,03,04 and 05 of the PL0158-23 protocol

Following the Johnson transformation, the particulate datasets exhibited approximately symmetric distributions, showing good agreement with the fitted normal curves despite the differences in sample size among the investigated protocol revisions.

Figure 4.33 presents the boxplot of transformed particulate contamination measured in the ISO 8 Clean Room environment.

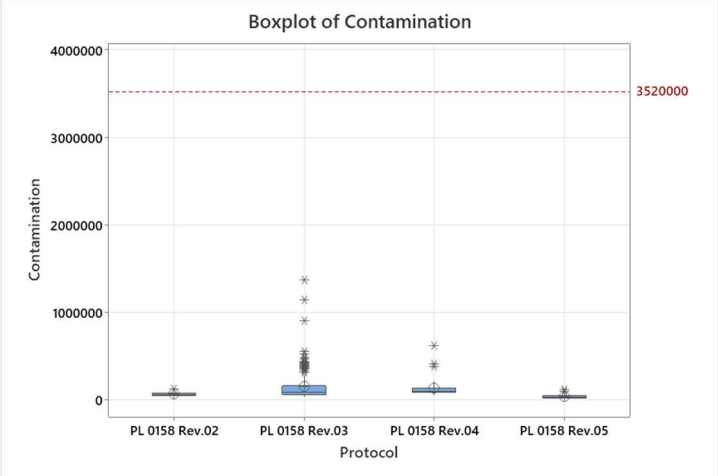
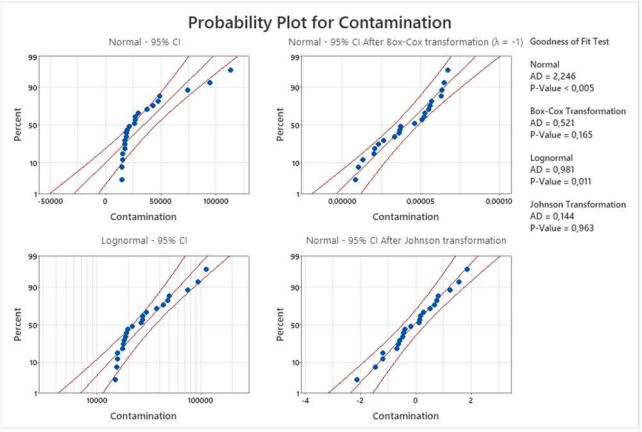


Figure 4.33 Boxplot of trasfromed particulate contamination collected in the ISO 8 Clean Room environment of the Via Statale facility for revision 02,03,04 and 05 of the PL0158-23 protocol

Revision 03 exhibited the greatest variability, with several high-value observations extending beyond the upper whisker. Revision 04 showed a lower overall dispersion, whereas Revision 05 was characterized by the narrowest distribution of measurements. Despite the presence of isolated high-value observations, all recorded particulate contamination values remained below the established acceptance limit.

Since the Johnson transformation was identified as the most suitable distribution for all protocol revisions, only the probability plot corresponding to Revision 05 is reported as a representative example in Figure 4.34.



Goodness of Fit Test

Distribution	AD	P
Normal	2,246	<0,005
Box-Cox Transformation	0,521	0,165
Lognormal	0,981	0,011
Johnson Transformation	0,144	0,963

Figure 4.34 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the ISO 8 Clean Room environment of the Via Statale facility for revision 05 of the PL0158 protocol,

As shown in Figure 4.34, the Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.144) and the highest p-value (0.963), indicating the best agreement between the transformed data and the theoretical normal distribution. Consequently, the Johnson transformation was selected for the subsequent statistical analyses.

The same statistical approach was subsequently applied to the Airlock environment.
Figure 4.35 shows the boxplot of transformed particulate contamination measured in the Air Lock environment of the Statale facility for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

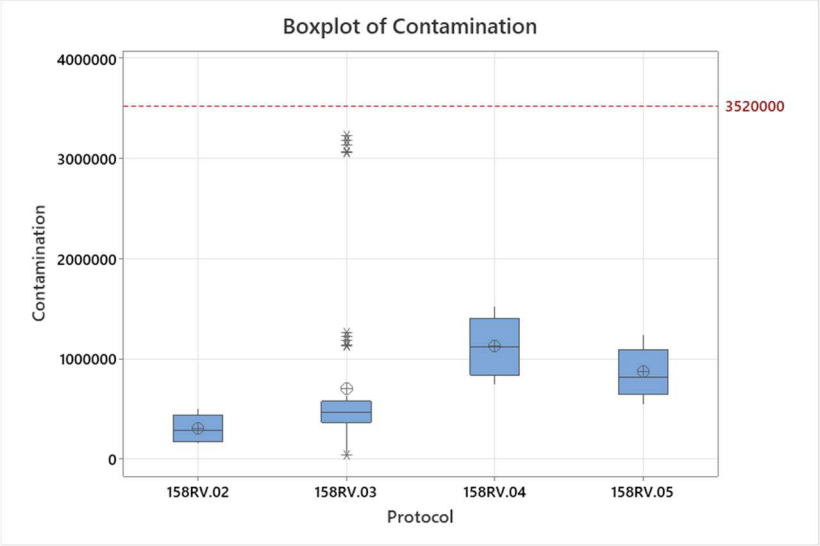
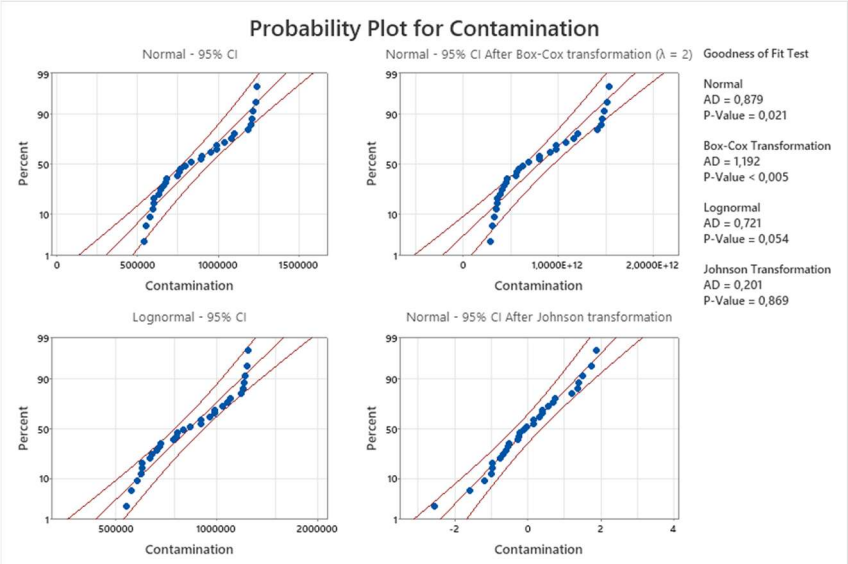


Figure 4.35 Boxplot representation of particulate contamination measured in the Airlock environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Figure 4.35 shows that Revision 03 exhibited the greatest data variability, with a wide interquartile range and several high-value observations. Revision 04 was characterized by the highest median particulate contamination, whereas Revision 05 showed a narrower distribution and lower overall variability. Despite these differences, all recorded particulate contamination values remained below the established acceptance limit throughout the investigated protocol revisions.

As the Johnson transformation consistently provided the best fit across all protocol revisions, only the corresponding probability plot for Revision 05 is presented in Figure 4.36.



Goodness of Fit Test

Distribution	AD	P
Normal	0,879	0,021
Box-Cox Transformation	1,192	<0,005
Lognormal	0,721	0,054
Johnson Transformation	0,201	0,869

Figure 4.36 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the Airlock environment of the Via Statale facility for revision 05 of the PL0158 protocol

As shown in Figure 4.36, the Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.201) and the highest p-value (0.869), indicating the best agreement between the transformed data and the theoretical normal distribution. Consequently, the Johnson transformation was selected for the subsequent statistical analyses.

Comparable analyses were also carried out for the Inlet Material Room environment across revisions 02, 03, 04, and 05 of the PL0158-23 protocol. Figure 4.37 shows the boxplot of transformed particulate contamination measured in the Inlet Materials Room environment of the Statale facility for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

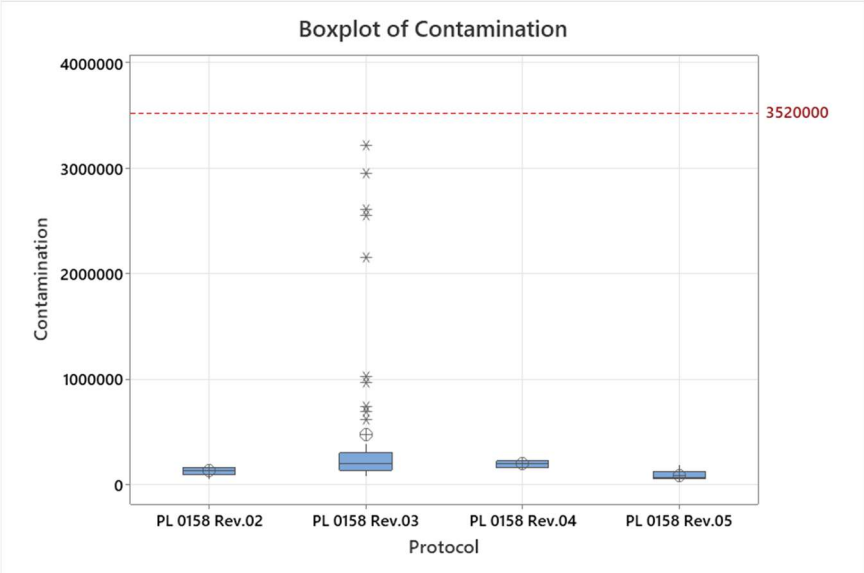


Figure 4.37 Boxplot representatiom collected in the Inlet Material Room environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158-23 protocol.

Revision 03 exhibited the greatest variability and was characterized by several isolated high-value observations, whereas Revision 04 showed a more compact distribution with a slightly higher median contamination level. Revision 05 exhibited the lowest contamination levels together with the narrowest data dispersion. Despite the presence of outliers, all recorded particulate contamination values remained below the established acceptance limit.

As the Johnson transformation consistently provided the best fit across all protocol revisions, only the corresponding probability plot for Revision 05 is presented in Figure 4.38.

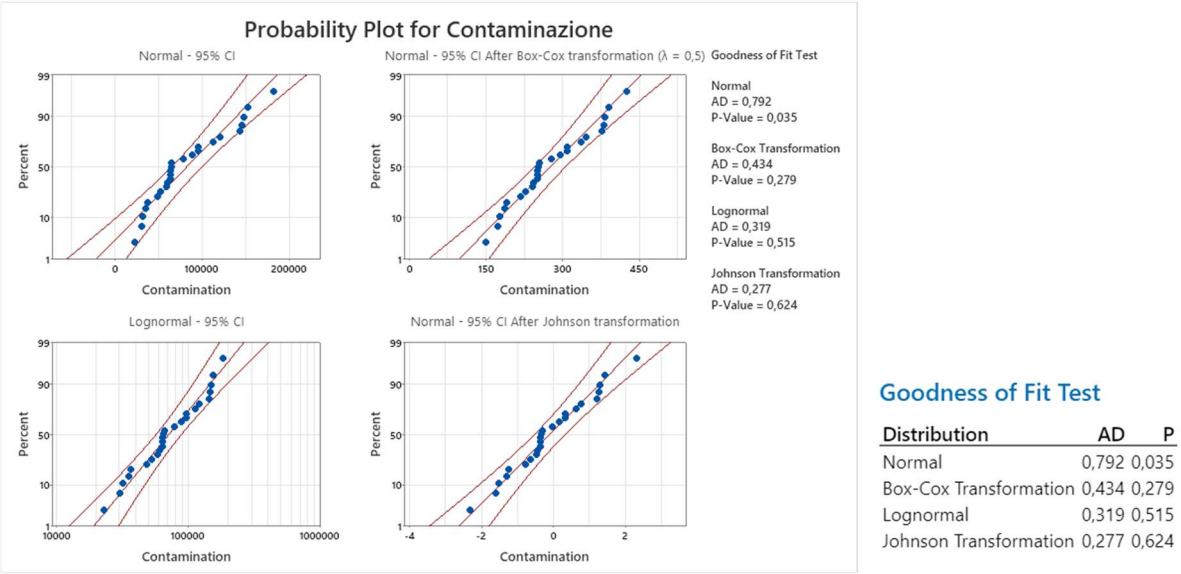


Figure 4.38 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the Inlet Material Room environment of the Via Statale facility for revision 05 of the PL0158 protocol.

As shown in Figure 4.38, the Johnson transformation yielded the lowest Anderson–Darling statistic (AD = 0.277) and the highest p-value (0.624), indicating the best agreement between the transformed data and the theoretical normal distribution. Consequently, the Johnson transformation was selected for the subsequent statistical analyses.

The Outlet Material Room was subsequently evaluated using the same statistical approach. Figure 4.39 presents the boxplot of particulate contamination measured in the Outlet Material Room of the Via Statale facility during Revisions 02, 03, 04, and 05 of the PL0158 protocol.

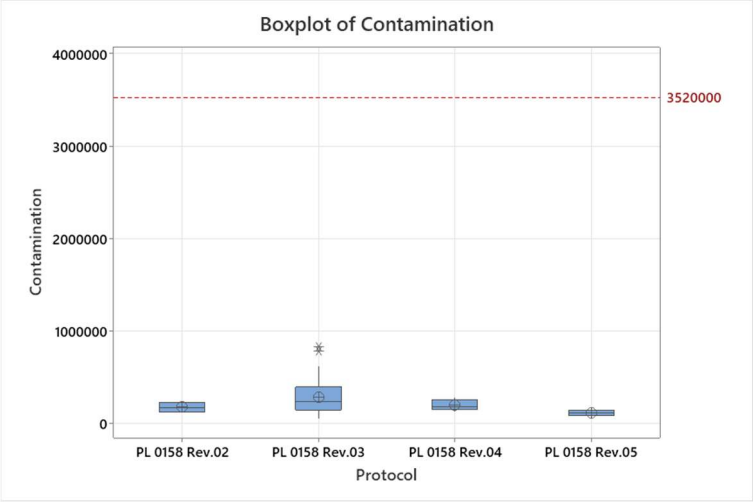
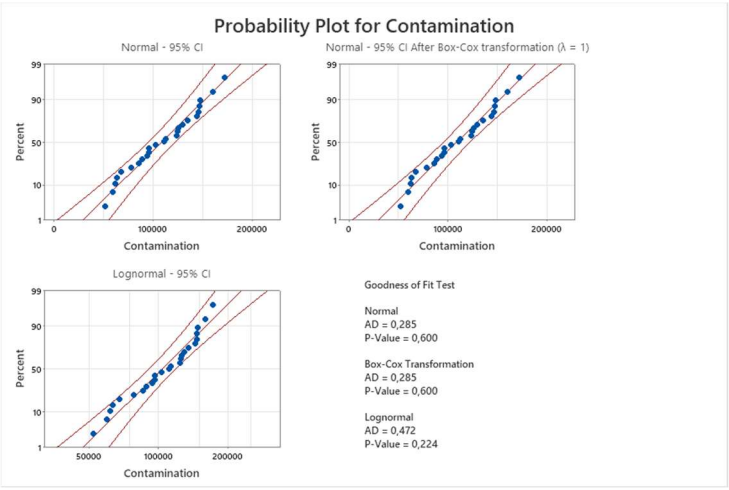


Figure 4.39 Boxplot representation collected in the Outlet Material Room environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Revision 03 exhibited the highest median particulate contamination together with the greatest data variability and a limited number of isolated high-value observations. In contrast, Revisions 02 and 04 showed intermediate contamination levels, whereas Revision 05 was characterized by the lowest median contamination value and the narrowest data distribution. Despite these differences, all recorded particulate contamination values remained below the established acceptance limit throughout the investigated protocol revisions.

Since both the normal distribution and the Box-Cox transformation provided an equally good fit for the particulate dataset collected during Revision 05, the corresponding probability plots and goodness-of-fit results are presented in Figure 4.40.



Goodness of Fit Test

Distribution	AD	P
Normal	0,285	0,600
Box-Cox Transformation	0,285	0,600
Lognormal	0,472	0,224

Figure 4.40 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the Outlet Material Room environment of the Via Statale facility for revision 05 of the PL0158 protocol.

The Distribution Identification analysis indicated that both the normal distribution and the Box-Cox transformation provided an equally satisfactory fit to the particulate dataset collected in the Outlet Material Room environment. In fact, both models yielded the same Anderson–Darling statistic (AD = 0.285) and p-value (0.600), indicating an equivalent agreement with the experimental data. In contrast, the lognormal distribution showed a lower goodness-of-fit.

Finally, the particulate distribution analysis was extended to the Airlock Dressing Room and Airlock Washing Room environments. Since these monitored areas were introduced only in Revision 05 of the PL0158 protocol, no comparison among protocol revisions could be performed. For completeness, the corresponding Distribution Identification analyses are reported in Appendix B.

Surface Microbiological Analysis

Table 4.5 reports the descriptive statistics of the surface microbiological measurements collected in the most representative environments of the Statale facility for the different revisions of the PL0158 protocol. The number of observations, mean values, and standard deviations are presented to provide an overall overview of the microbiological contamination levels prior to the graphical analysis.

Table 4.5 Descriptive statistical analysis of surface microbiological measurements collected in the monitored environments of the Statale facility.

ID	ROOM	ISO	PL0158 Rev.	N	Mean	St.Dv.
1	Clean room	7	05	20	5,85	9,16
		8	02	12	10	11
			03	66	6,3	8,4
			04	20	9,4	12
			05	30	7,2	12,52
2	Inlet Materials room	8	04	10	9	14
			05	25	5,12	3,98
3	Outlet Materials room	8	04	10	2,8	2
			05	25	9,08	8,81
7	Airlock	8	02	2	4,5	3,54
			03	11	11	10,36
			04	10	4,4	3,84
			05	25	8,52	8,03
5	Airlock Dressing room	8	05	25	11,56	8,95
4	Airlock Washing room	8	05	25	5,68	6,82

The graphical analyses presented below refer to the most representative environments of the Via Statale facility across Revisions 02, 03, 04, and 05 of the PL0158 protocol. Boxplots were used to compare microbiological contamination levels and data variability among the different protocol revisions.

The analysis begins with the Clean Room environment. Figure 4.41 and Figure 4.42 present the boxplots of surface microbiological contamination measured on the remaining monitored surfaces and on the floor, respectively, during Revisions 02, 03, 04, and 05 of the PL0158 protocol. Since the acceptance limits differ between floor samples and the remaining monitored surfaces, the corresponding results are presented separately.

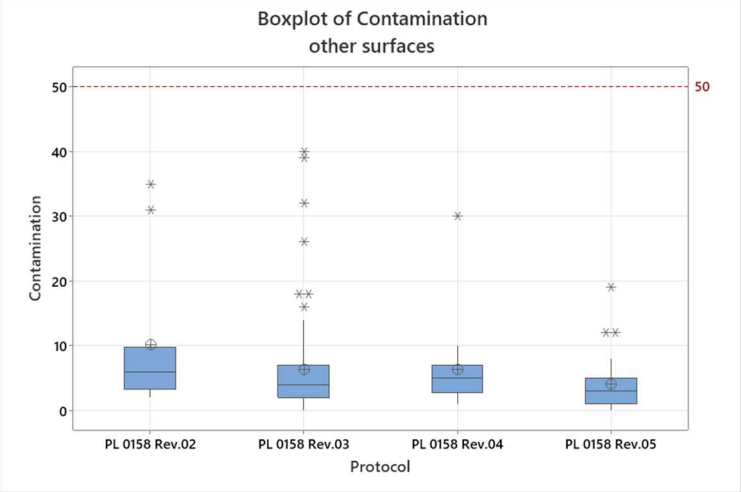


Figure 4.41 Boxplot representation of surface microbiological of contamination of other surfaces measured in the ISO 8 Clean Room environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol

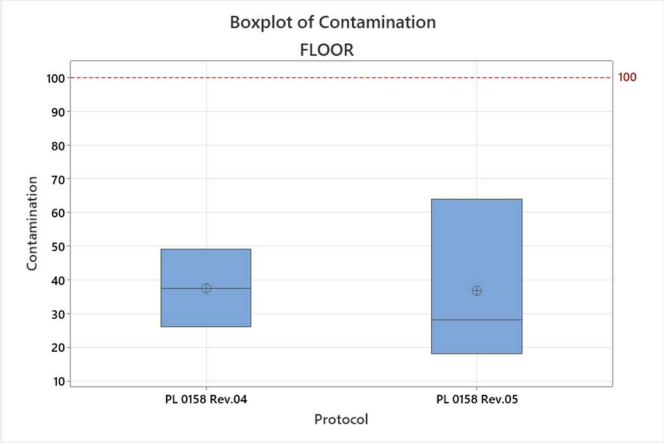


Figure 4.42 Boxplot representation of surface microbiological contamination of the floor measured in the ISO 8 Clean Room environment of the Via Statale facility for revisions 04 and 05 of the PL0158 protocol

Overall, the microbiological contamination remained low throughout the investigated protocol revisions. Floor samples exhibited limited variability, whereas the remaining monitored surfaces showed a wider distribution of observations together with a small number of isolated outliers. Nevertheless, all recorded values complied with the corresponding acceptance limits.

The same descriptive graphical analyses were subsequently performed for the Inlet Material Room and Outlet Material Room environments.
Figure 4.43 presents the boxplot of surface microbiological contamination measured in the Inlet Materials Room environment for Revisions 04 and 05 of the PL0158 protocol.

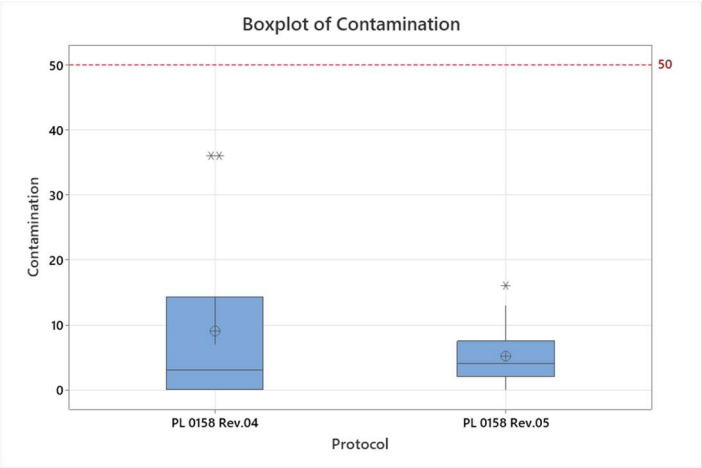


Figure 4.43 Boxplot representation of surface microbiological contamination collected in the Inlet Material Room environment of the Via Statale facility for revisions 04 and 05 of the PL0158 protocol

Compared with Revision 04, Revision 05 exhibited a narrower data distribution and lower overall variability. Although isolated outliers were observed in both revisions, all microbiological contamination values remained below the acceptance limit.

Figure 4.44 presents the boxplot of surface microbiological contamination measured in the Outlet Material Room during Revisions 04 and 05 of the PL0158 protocol.

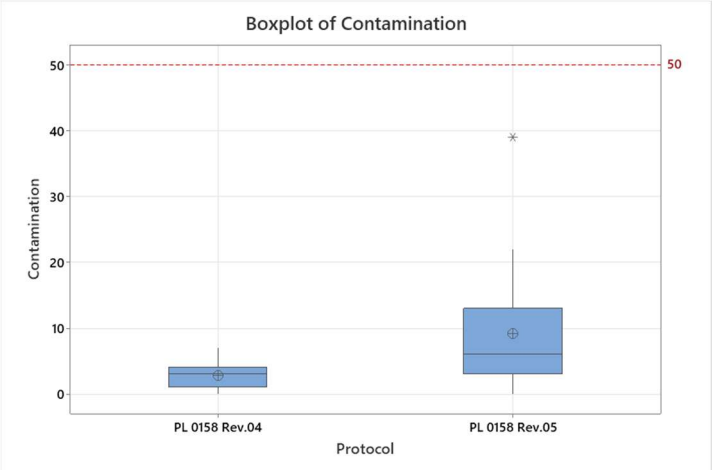


Figure 4.44 Boxplot representation of surface microbiological contamination collected in the Outlet Material Room environment of the Via Statale facility for revisions 04 and 05 of the PL0158 protocol

Compared with Revision 04, Revision 05 showed higher median contamination values together with a broader distribution of the measurements. Although an isolated outlier was observed in Revision 05, all recorded microbiological contamination values complied with the corresponding acceptance limit.

Finally, the same descriptive graphical analyses were applied to the Airlock environment across Revisions 02, 03, 04, and 05 of the PL0158 protocol.
Figure 4.45 presents the boxplot of surface microbiological contamination measured in the Airlock environment for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

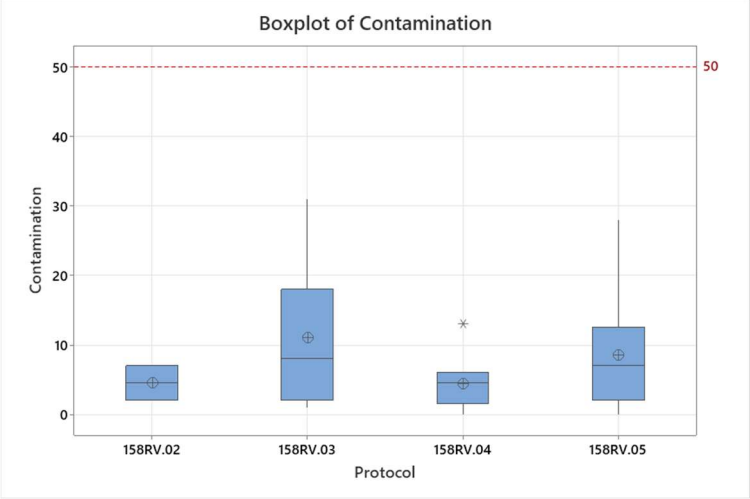


Figure 4.45 Boxplot representation of surface microbiological contamination collected in the Airlock environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Revision 03 exhibited the greatest variability together with the highest median contamination level among the investigated revisions. In contrast, Revision 04 showed a more compact distribution with generally lower contamination values, whereas Revision 05 displayed intermediate variability. Overall, all recorded microbiological contamination values complied with the established acceptance limit.

Finally, the same descriptive graphical approach was applied to airborne microbiological monitoring data collected in the selected environments of the Via Statale facility across the available revisions of the PL0158 protocol.

Airborne Microbiological Analysis

Table 4.6 summarizes the descriptive statistics of the airborne microbiological measurements collected in the most representative monitored environments of the Statale facility across the different revisions of the PL0158 protocol. For each monitored environment, the table reports the number of observations (N), the mean contamination value, and the corresponding standard deviation, providing an overview of the airborne microbiological contamination levels prior to the graphical analyses presented in the following sections.

Table 4.6 Mean values and standard deviations associated with airborne microbiological measurements collected in the monitored environments.

ID	ROOM	ISO	PL0158 Rev.	N	Mean	St.Dv.
1	Clean room	7	05	14	37,14	23
		8	02	20	23,5	21,34
			03	110	38,1	32,6
			04	14	62,86	45,8
			05	21	30,9	30,1
2	Inlet Materials room	8	02	2	35	21
			03	11	43,6	22,5
			04	4	80,25	56,747
			05	10	66	27,2
3	Outlet Materials room	8	02	2	20	0
			03	11	72,73	38,5
			04	4	122,5	22,174
			05	10	62	43,4
7	Airlock	8	02	2	75	21,21
			03	12	114	44,6
			04	4	72	58,67
			05	10	110	55,38
5	Airlock Dressing room	8	05	10	92,6	41,2
4	Airlock Washing room	8	05	10	67	42,44

The graphical analyses presented below refer to the most representative environments of the Via Statale facility across the available revisions of the PL0158 protocol.

The Clean Room environment is illustrated through both histogram and boxplot analyses, whereas the remaining monitored environments are presented by means of boxplots to facilitate the comparison of airborne microbiological contamination levels and data variability among protocol revisions.

The analysis begins with the Clean Room environment. Since this area was reclassified from ISO 8 to ISO 7 in Revision 05 of the PL0158 protocol, the results are presented separately according to the corresponding ISO classification.

Figure 4.48 presents the histogram of airborne microbiological contamination measured in the Clean Room when classified as ISO 7 during Revision 05.

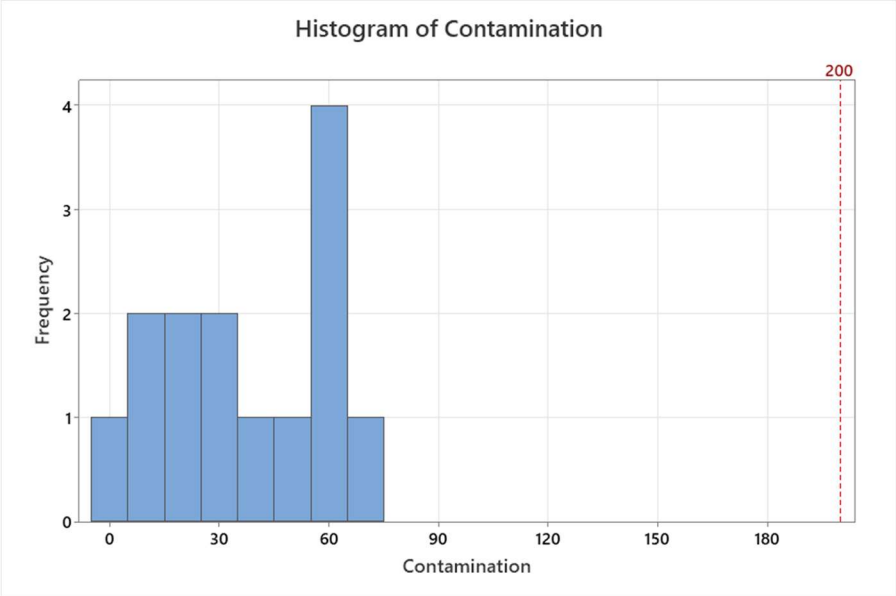


Figure 4.46 Histogram of airborne microbiological contamination collected in the ISO 7 Clean Room environment of the Via Statale facility according to the different revisions of the PL0158 protocol

The measurements were mainly concentrated at low and intermediate contamination levels, and all recorded values remained well below the established acceptance limit of 200 CFU.

Figure 4.49 shows the histogram of airborne microbiological contamination measured in the ISO 7 Clean Room environment for Revision 05 of the PL0158 protocol.

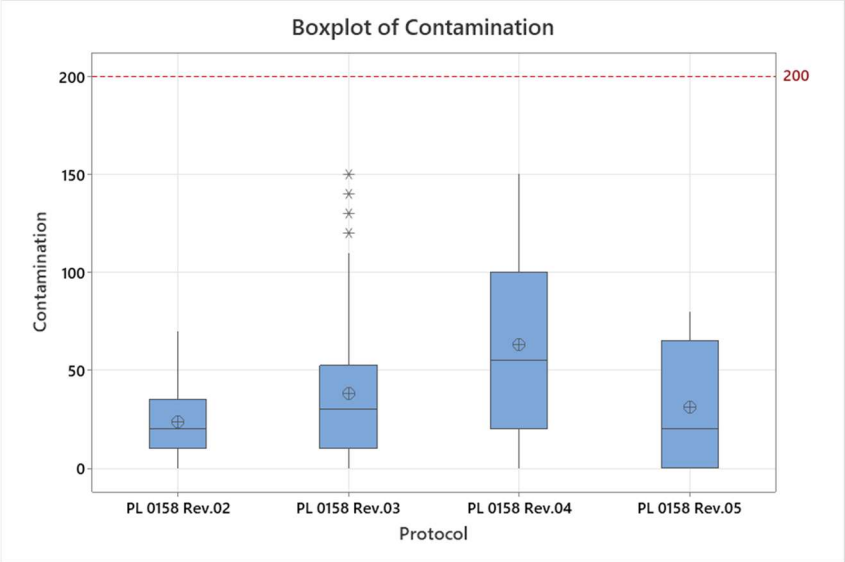


Figure 4.47 Boxplot representation of airborne microbiological contamination collected in the ISO 8 Clean Room environment of the Via Statale facility according to the different revisions of the PL0158 protocol.

Revision 04 exhibited the highest median contamination level together with the greatest data variability, whereas Revision 02 showed the lowest contamination levels. Revisions 03 and 05 displayed intermediate distributions. Despite these differences, all recorded airborne microbiological contamination values complied with the established acceptance limit.

The same descriptive graphical analyses were subsequently performed for the Inlet Material Room and Outlet Material Room environments.

Figure 4.50 presents the boxplot of airborne microbiological contamination measured in the Inlet Material Room environment for Revisions 02, 03, 04, and 05 of the PL0158 protocol

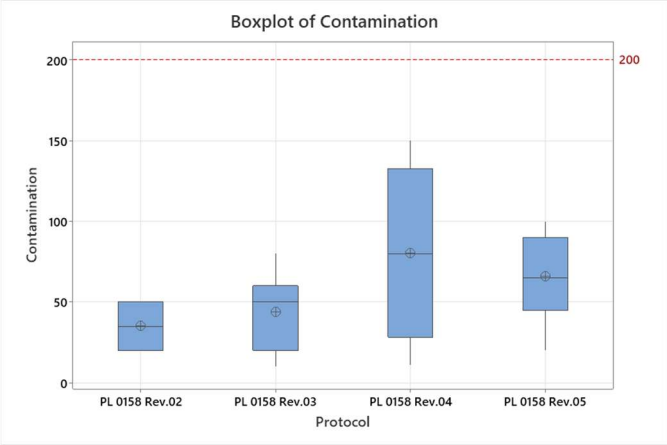


Figure 4.48 Box plot representation of airborne microbiological contamination collected in the Inlet Material Room environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Revision 04 exhibited the highest contamination levels together with the greatest variability, whereas Revisions 02 and 03 showed lower contamination levels and a more compact distribution of the measurements. Revision 05 displayed intermediate contamination values with reduced variability compared with Revision 04. Overall, all recorded microbiological contamination values complied with the established acceptance limit.

Figure 4.51 presents the boxplot of airborne microbiological contamination measured in the Outlet Material Room environment for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

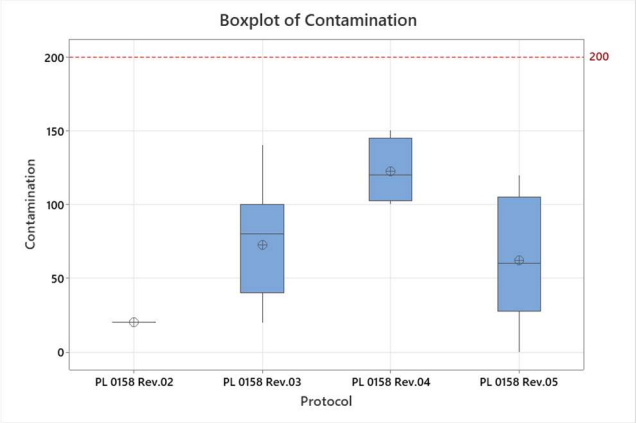


Figure 4.49 Boxplot representation of airborne microbiological contamination collected in the Outlet Material Room environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Revision 04 showed the highest contamination levels, whereas Revision 02 was characterized by the lowest recorded values. Revisions 03 and 05 exhibited intermediate contamination levels together with a broader distribution of the measurements. Nevertheless, all recorded airborne microbiological contamination values complied with the established acceptance limit.

Finally, the same descriptive graphical analyses were applied to the Airlock environment across Revisions 02, 03, 04, and 05 of the PL0158 protocol.

Figure 4.52 presents the boxplot of airborne microbiological contamination measured in the Airlock environment for Revisions 02, 03, 04, and 05 of the PL0158 protocol.

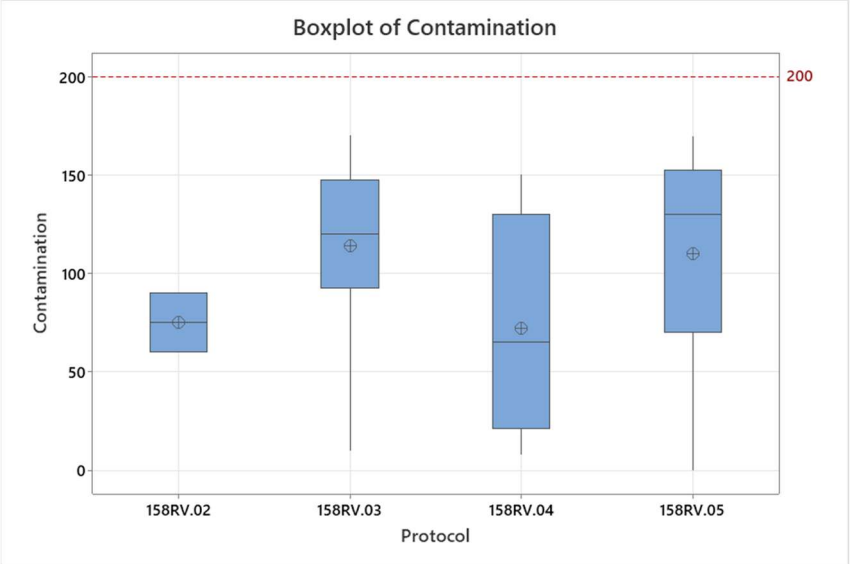


Figure 4.50 Boxplot representation of airborne microbiological contamination collected in the Airlock environment of the Via Statale facility for revisions 02, 03, 04, and 05 of the PL0158 protocol.

Revision 02 exhibited the lowest variability, whereas Revisions 03 and 05 showed higher contamination levels together with a broader distribution of the measurements. Revision 04 was characterized by the greatest overall data variability. Nevertheless, all recorded airborne microbiological contamination values complied with the established acceptance limit.

4.2 Process Variability

In this subsection, the temporal variability of the monitored processes is evaluated using Individual Control Charts (I-Charts). The analysis was performed on the most representative environments of both the Bove and Via Statale facilities, considering particulate, surface microbiological, and airborne microbiological monitoring data.

I-Charts were used to assess process stability over time by identifying trends, variability, and potential deviations from the expected process behaviour within the controlled environments.

4.2.1 Via Bove facility

The following I-Charts refer to particulate, surface microbiological, and airborne microbiological monitoring performed in the most representative environments of the Bove facility. Since the Clean Room represents the most critical production environment, both particulate and microbiological process variability are discussed in the main text. For the remaining monitored environments, only particulate I-Charts are presented, whereas the corresponding surface microbiological I-Charts are reported in Appendix A for completeness.

The analysis begins with the Clean Room environment. Figure 4.53 presents the Individual Control Chart of particulate contamination measured during Revisions 02, 04, and 05 of the PL048-23 protocol.

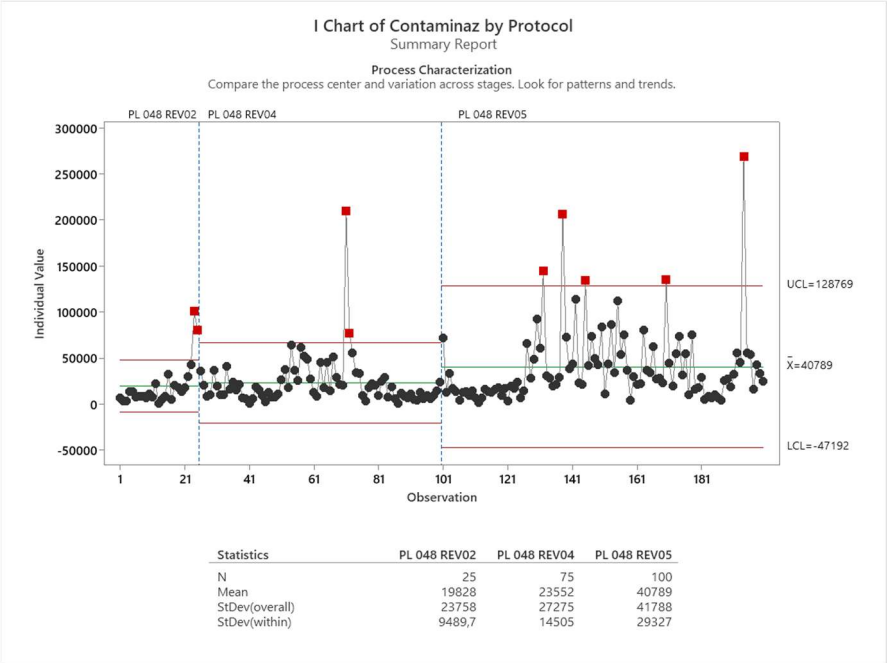


Figure 4.51 I-Chart of particulate measurements collected in the Clean room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Compared with Revision 02 (mean = 19,828 particles), Revisions 04 (mean = 23,552 particles) and 05 (mean = 40,789 particles) exhibited progressively higher average contamination levels and greater variability. In addition, several isolated outliers were identified throughout the monitoring period, with a higher frequency and magnitude in Revisions 04 and 05, where higher contamination peaks were observed.

Figure 4.54 presents the corresponding Individual Control Chart for surface microbiological contamination.

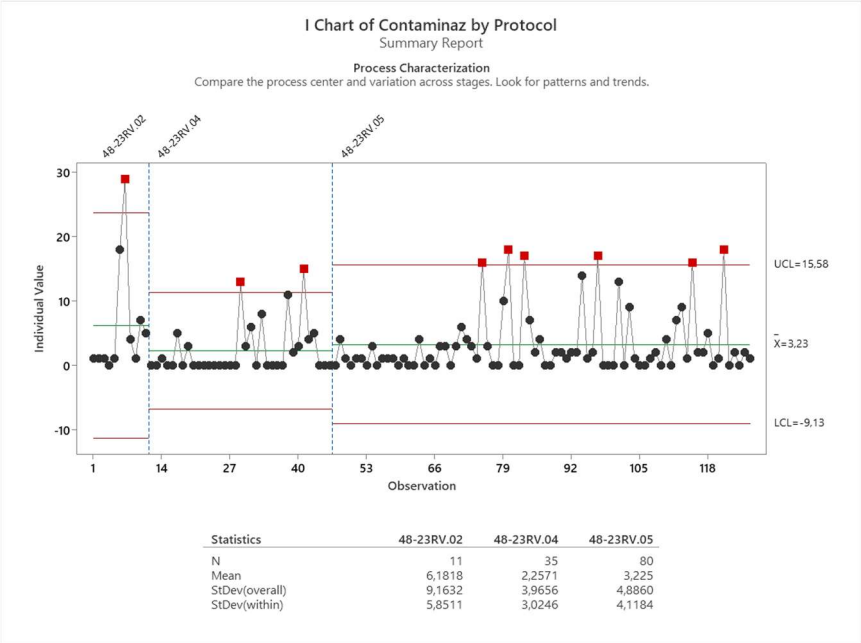


Figure 4.52 I-Chart of surface microbiological measurements collected in the Clean room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 04 (mean = 2.26 CFU) exhibited the lowest average contamination level together with the most compact distribution of the measurements. Revision 05 showed a slightly higher average contamination level (mean = 3.23 CFU) and several isolated outliers. Although Revision 02 displayed the highest average contamination level (mean = 6.18 CFU), this result was based on a limited number of observations (N = 11). Overall, all recorded microbiological contamination values remained below the established acceptance limit (75 CFU).

The temporal variability of airborne microbiological contamination is illustrated in Figure 4.55.

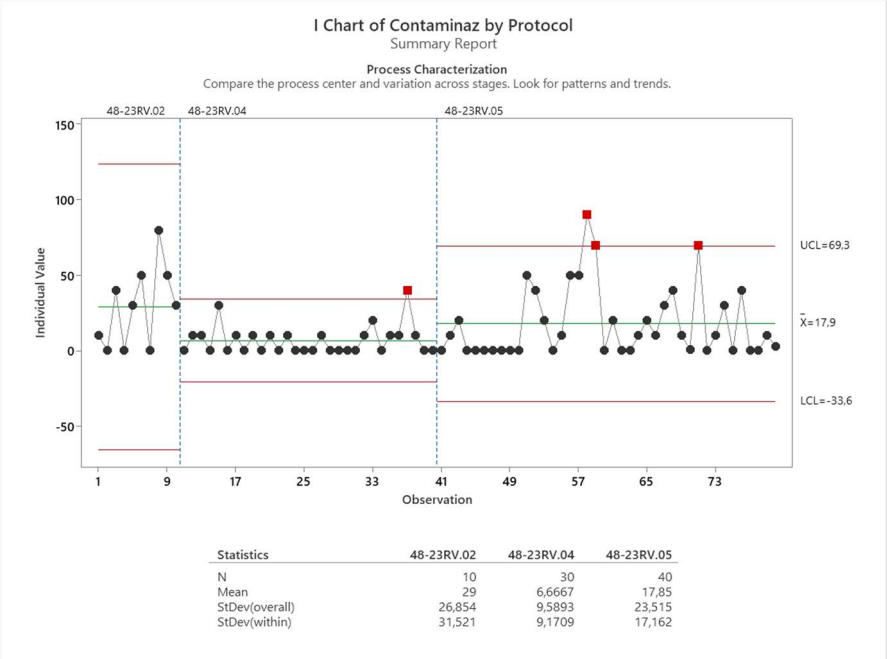


Figure 4.53 I-Chart of airborne microbiological measurements collected in the Clean room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol

The chart highlights differences in both the process mean and the variability among the investigated revisions. Revision 04 exhibited the lowest average contamination level (6.67 CFU) together with the most compact distribution of the measurements, whereas Revision 05 showed a higher average value (17.85 CFU) and greater variability, with several isolated outliers. Although Revision 02 displayed the highest average contamination level (29.00 CFU), this result was based on a limited number of observations (N = 10). Overall, all recorded microbiological contamination values remained below the established acceptance limit (200 CFU).

The same analysis was subsequently applied to the Airlock environment. Figure 4.56 presents the corresponding I-Chart.

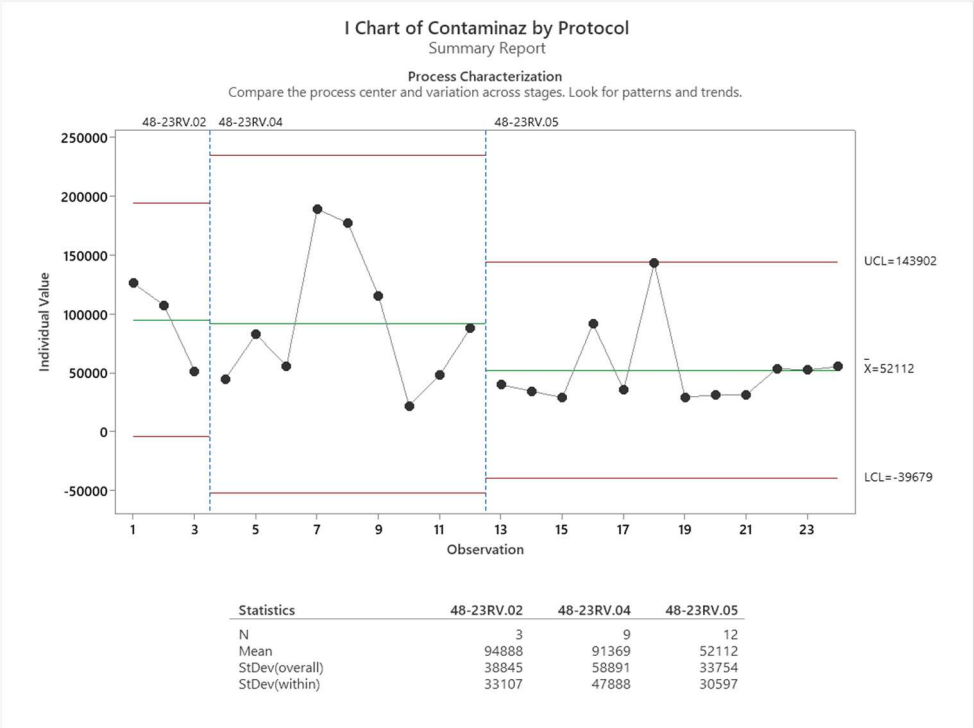


Figure 4.54 I-Chart of particulate measurements collected in the Airlock environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Compared with Revision 02 (mean = 94,888 particles), Revision 04 exhibited a slightly lower average contamination level (mean = 91,369 particles) together with greater variability. Revision 05 showed the lowest average contamination level (mean = 52,112 particles) and a more compact distribution of the measurements. Overall, no pronounced outliers were observed during the monitoring period.

The Incoming Control Room was subsequently evaluated using the same approach. Figure 4.57 presents the corresponding I-Chart.

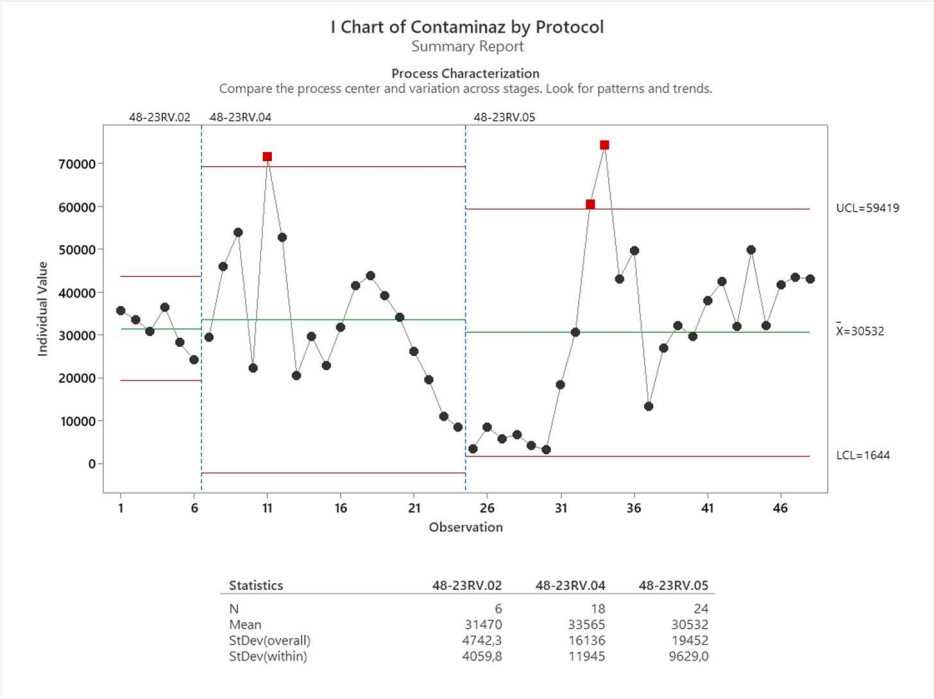


Figure 4.55 I-Chart of particulate measurements collected in the Incoming Control Room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

The process mean remained relatively stable throughout the three monitoring periods (31,470, 33,565, and 30,532 particles for Revisions 02, 04, and 05, respectively). Revision 04 exhibited the highest variability (StDev = 16,136 particles) together with two observations exceeding the upper control limit, whereas Revision 05 showed lower within-process variability (StDev = 19,452 particles; within StDev = 9,629 particles) despite the presence of two isolated outliers. Overall, the chart suggests a generally stable process with limited isolated variability events.

Figure 4.58 presents the Individual Control Chart obtained for the Incoming Material Room.

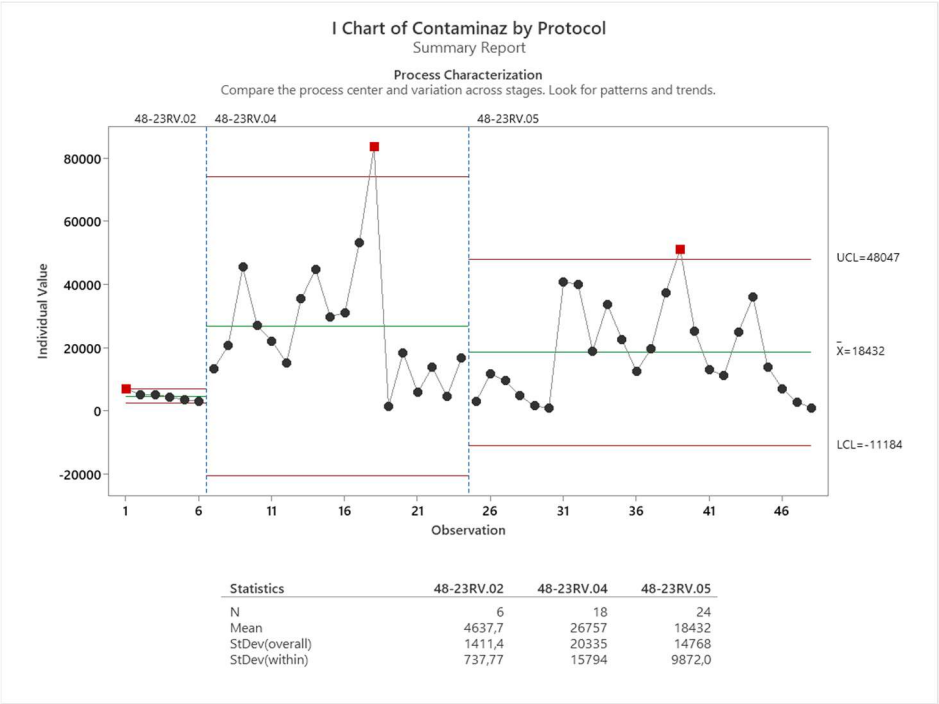


Figure 4.56 I-Chart of particulate measurements collected in the Incoming Material Room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 04 exhibited the highest average contamination level (mean = 26,757 particles) together with the greatest variability and several isolated outliers. Compared with Revision 04, Revision 05 showed a lower average contamination level (mean = 18,432 particles) and a more compact distribution of the measurements, although isolated outliers were still observed. Revision 02 was characterized by the lowest average contamination level (mean = 4,638 particles) and the smallest data dispersion.

The same analysis was subsequently performed for the Manufacturing Cassette environment (Figure 4.59).

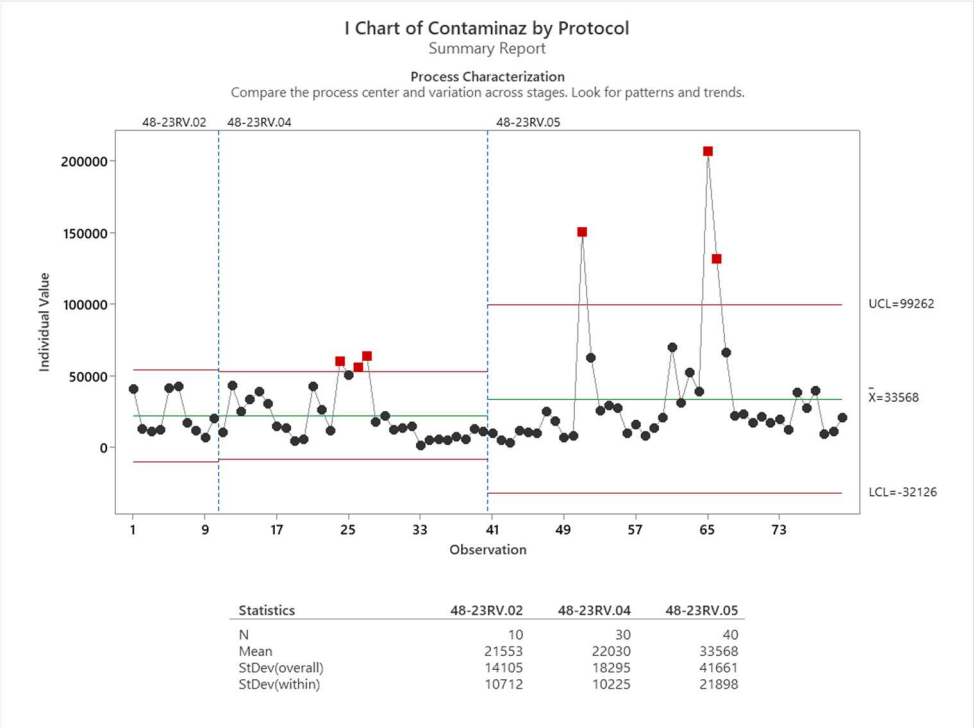


Figure 4.57 I-Chart of particulate measurements collected in the Manufacturing Cassette environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Revisions 02 and 04 exhibited comparable average contamination levels, with mean values of 21,553 particles and 22,030 particles, respectively. In contrast, Revision 05 showed the highest average contamination level (mean = 33,568 particles) together with greater variability and several isolated outliers, including the highest contamination peaks observed during the monitoring period.

Finally, the temporal variability of particulate contamination was evaluated for the Women's and Men's Changing Rooms, as shown in Figures 4.60 and 4.61.

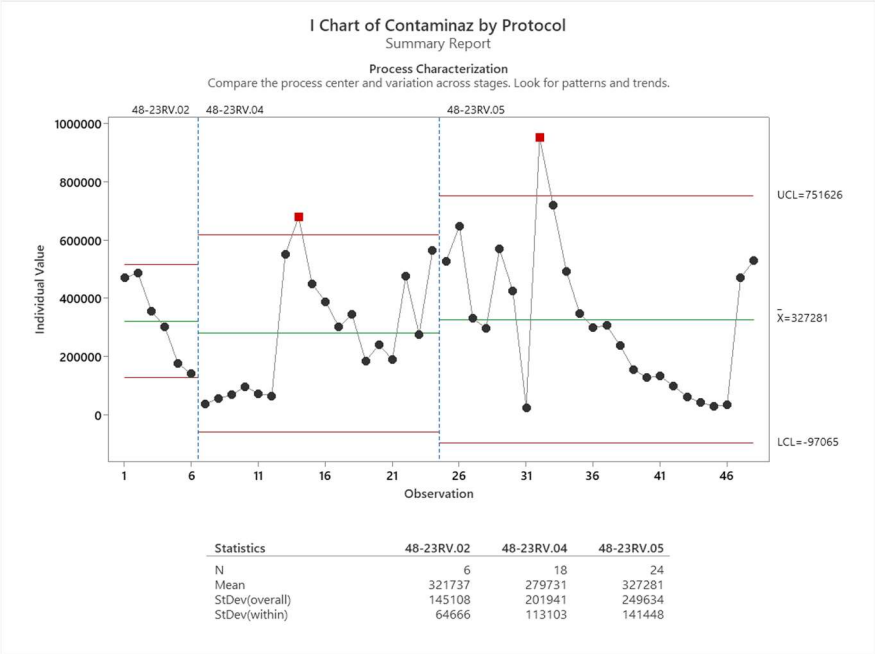


Figure 4.58 I-Chart of particulate measurements collected in the Women's Changing Room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Revisions 02 and 05 exhibited comparable average contamination levels, with mean values of 321,737 particles and 327,281 particles, respectively. In contrast, Revision 04 showed a lower average contamination level (mean = 279,731 particles). Revision 05 also exhibited greater process variability together with several isolated outliers, including the highest contamination peak observed among the investigated revisions.

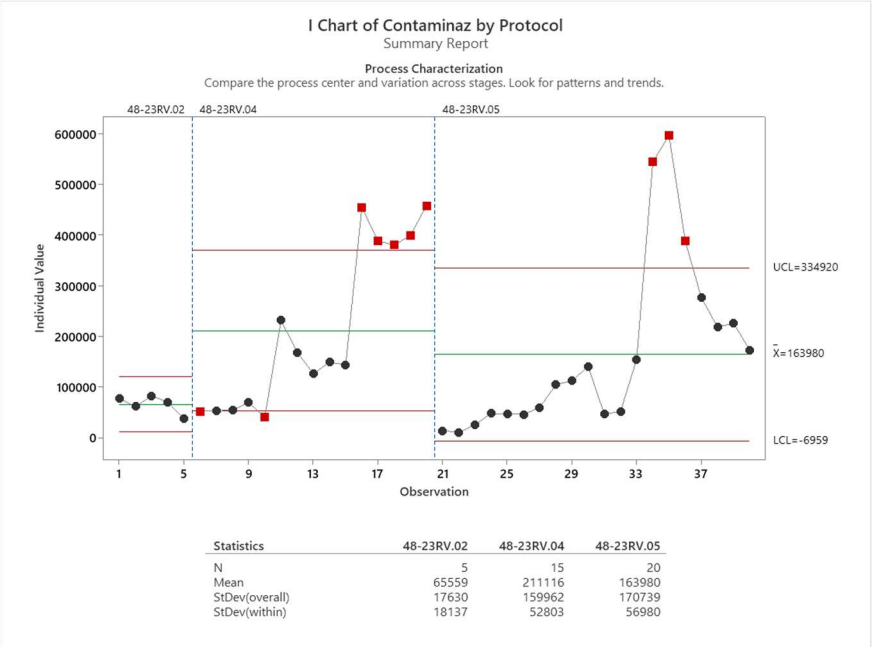


Figure 4.59 I-Chart of particulate measurements collected in the Men's Changing Room environment of the Bove facility across revisions 02, 04, and 05 of the PL048-23 protocol.

Revision 04 exhibited the highest average contamination level (mean = 211,116 particles), followed by Revision 05 (mean = 163,980 particles), whereas Revision 02 showed the lowest average contamination level (mean = 65,559 particles). Revisions 04 and 05 also displayed greater process variability together with several isolated outliers, while Revision 02 was characterized by a lower average contamination level and a more stable distribution of the measurements.

4.2.2 Via Statale facility

The following I-Charts illustrate the temporal variability of particulate, surface microbiological, and airborne microbiological contamination measured in the most representative environments of the Via Statale facility. Since the Clean Room represents the most critical production environment, particulate, surface microbiological, and airborne microbiological process variability are discussed in the main text. For the remaining monitored environments, only particulate I-Charts are presented, whereas the corresponding surface microbiological I-Charts are reported in Appendix B for completeness.

Particular attention was dedicated to the Clean Room environment, which was reclassified from ISO 8 to ISO 7 in the latest revision of the PL0158 protocol. Consequently, separate I-Charts are presented for the two ISO classifications in order to describe the temporal variability of particulate, surface microbiological, and airborne microbiological contamination.

The analysis begins with the ISO 7 Clean Room. Figure 4.62 presents the corresponding Individual Control Chart.

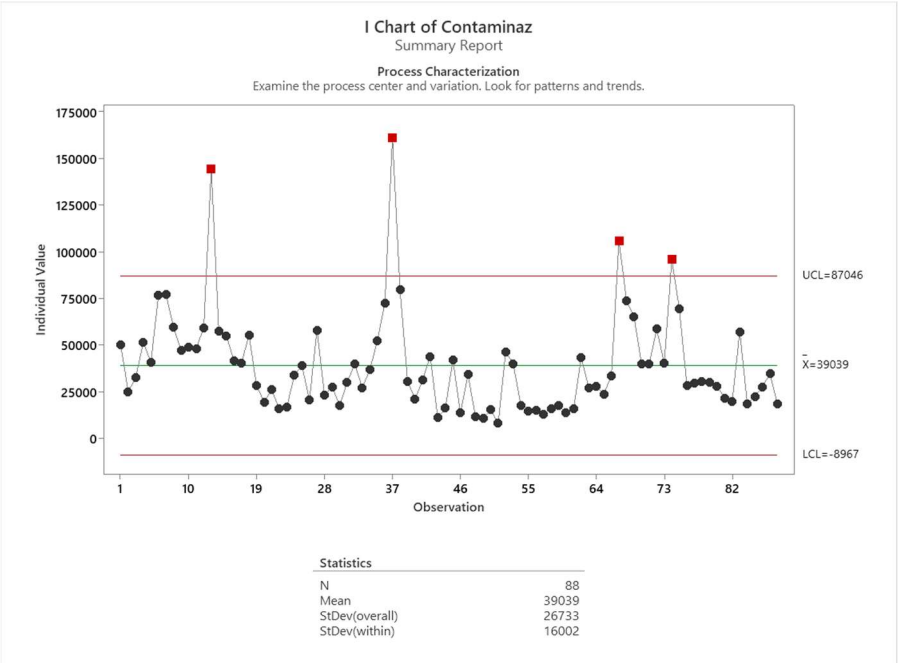


Figure 4.60 I-Chart of particulate measurements collected in the ISO 7 Clean Room environment of the Via Statale facility during Rev.05 of the PL0158 protocol.

The average particulate contamination was 39,039 particles, with an overall standard deviation of 26,733 particles. Most observations were distributed around the process mean, although several isolated outliers exceeding the upper control limit were identified, indicating occasional contamination peaks during the monitoring period. Despite these isolated events, the majority of the measurements remained within the established control limits.

The same environment was subsequently evaluated considering the monitoring period in which it was classified as ISO 8. Figure 4.63 presents the corresponding Individual Control Chart.

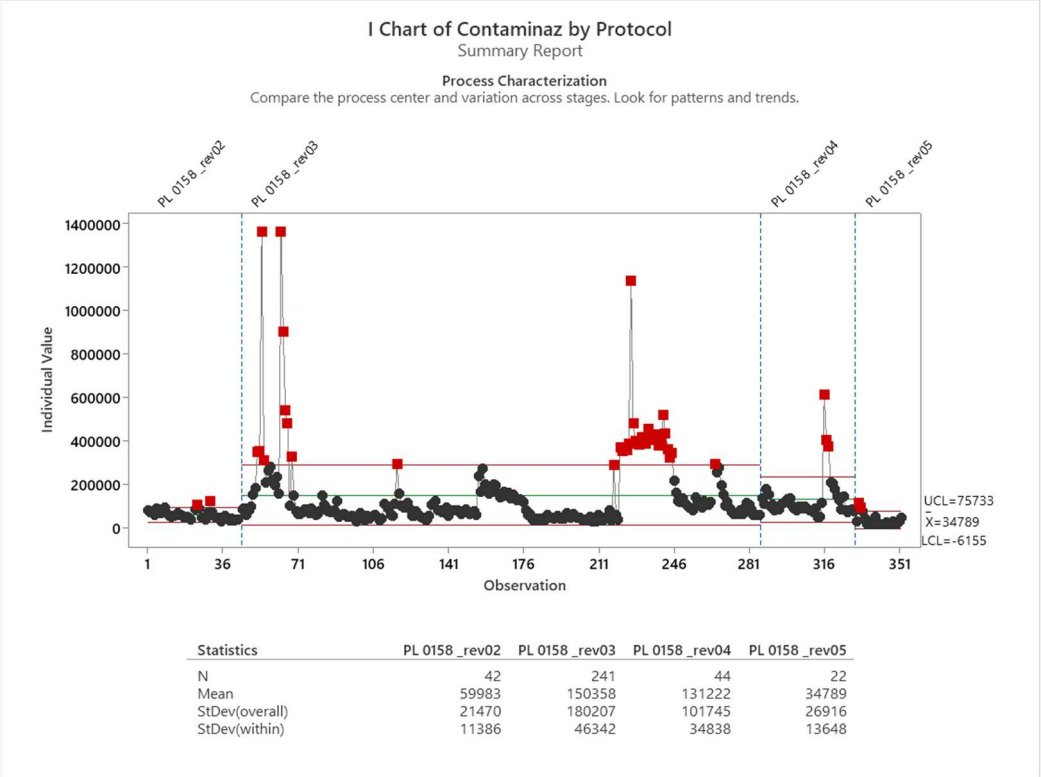


Figure 4.61 I-Chart of particulate measurements collected in the ISO 8 Clean Room environment of the Via Statale facility across revisions 02, 03, 04 and 05 of the PL0158 protocol.

Revision 03 exhibited the highest average contamination level (mean = 150,358 particles) together with the greatest process variability and numerous isolated outliers. Revision 04 showed a lower average contamination level (mean = 131,222 particles) but maintained a high degree of variability, with several outliers still exceeding the upper control limit. In contrast, Revision 05 was characterized by the lowest average contamination level (mean = 34,789 particles) and a substantial reduction in process variability. Revision 02 exhibited an intermediate average contamination level (mean = 59,983 particles) with fewer isolated outliers than Revisions 03 and 04.

Figure 4.64 illustrates the temporal variability of airborne microbiological contamination measured in the ISO 8 Clean Room.

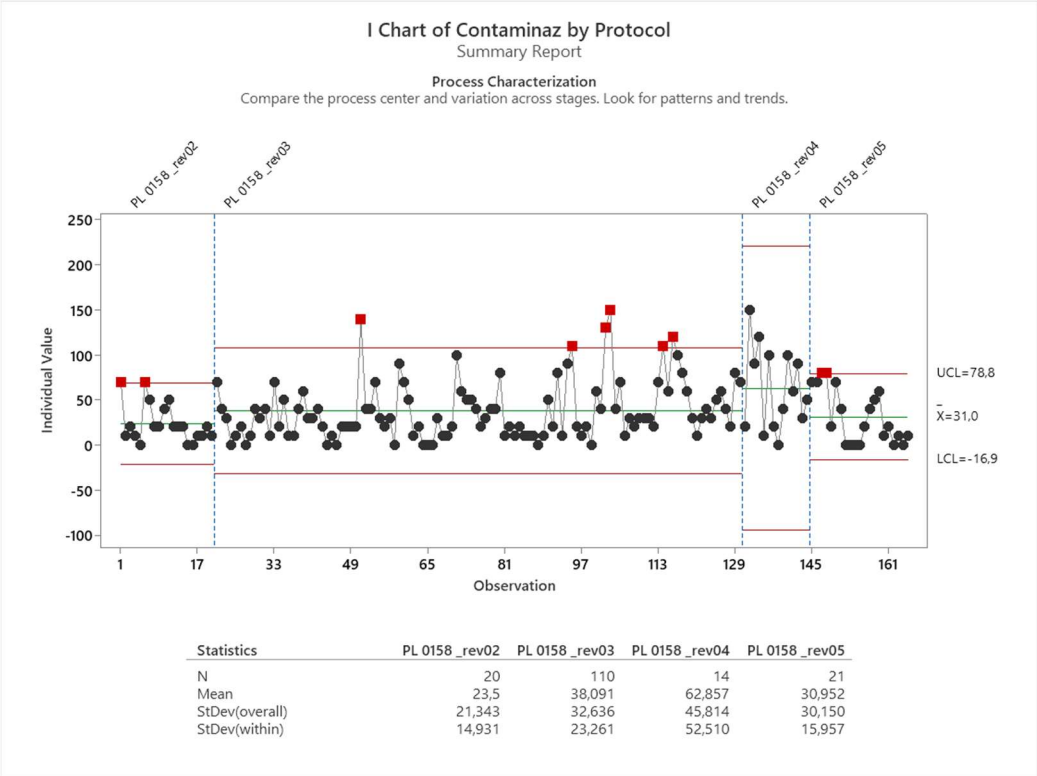


Figure 4.62 I-Chart of airborne microbiological measurements collected in the ISO 8 Clean Room environment of the Via Statale facility across revisions 02, 03, 04 and 05 of the PL0158 protocol.

Revision 04 exhibited the highest average contamination level (mean = 62.9 CFU/m³) together with the greatest process variability (StDev = 45.8 CFU/m³) and several isolated outliers. Revision 03 showed an intermediate average contamination level (mean = 38.1 CFU/m³), whereas Revision 02 presented the lowest mean value (23.5 CFU/m³) and a more stable distribution of the measurements. In Revision 05, the average contamination level decreased to 31.0 CFU/m³, accompanied by a reduction in process variability (StDev = 30.2 CFU/m³) and fewer outliers compared with Revision 04.

Figure 4.65 presents the Individual Control Chart of surface microbiological contamination measured in the ISO 8 Clean Room

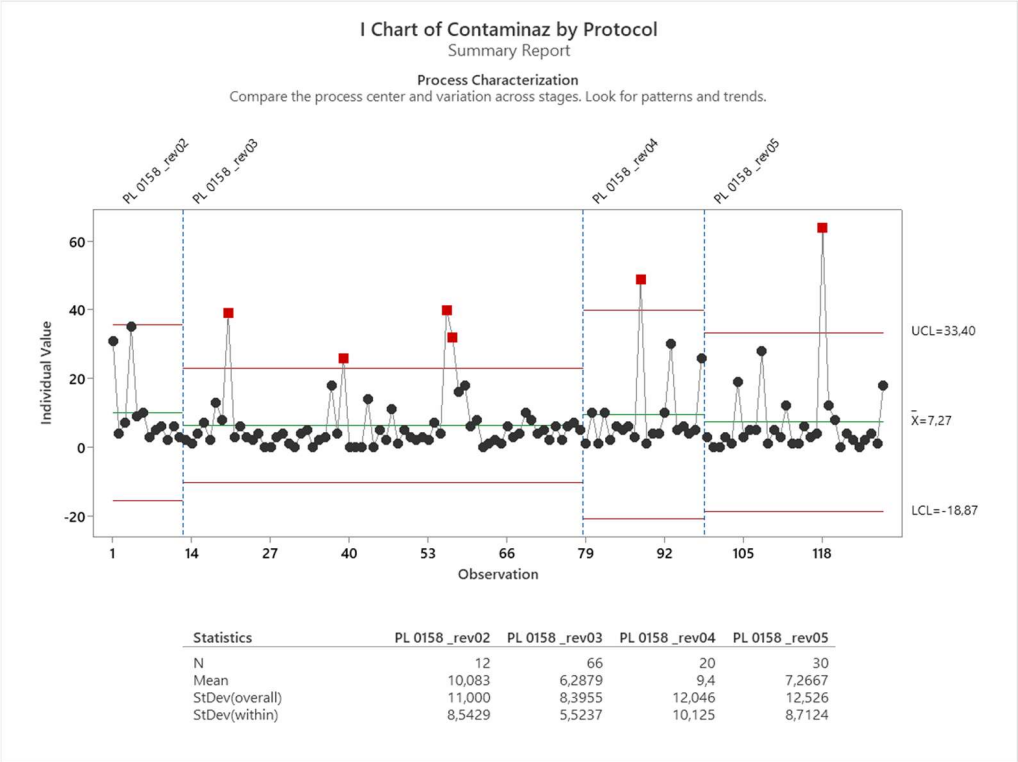


Figure 4.63 I-Chart of surface microbiological measurements collected in the ISO 8 Clean Room environment of the Via Statale facility across revisions 02, 03, 04 and 05 of the PL0158 protocol

The average contamination levels remained relatively comparable throughout the investigated revisions, ranging from 6.3 CFU/plate (Revision 03) to 10.1 CFU/plate (Revision 02). Process variability was also similar among the revisions, although Revisions 04 and 05 exhibited slightly higher standard deviations (12.0 and 12.5 CFU/plate, respectively) than Revisions 02 (11.0 CFU/plate) and 03 (8.4 CFU/plate). Several isolated outliers were identified across all revisions, with the highest contamination peak observed during Revision 05.

The temporal variability analysis was subsequently extended to the Airlock, Airlock Dressing Room, and Airlock Washing Room environments. Since only particulate monitoring data were considered in the main text for these areas, the corresponding Individual Control Charts are presented in Figures 4.66–4.67-4.68.

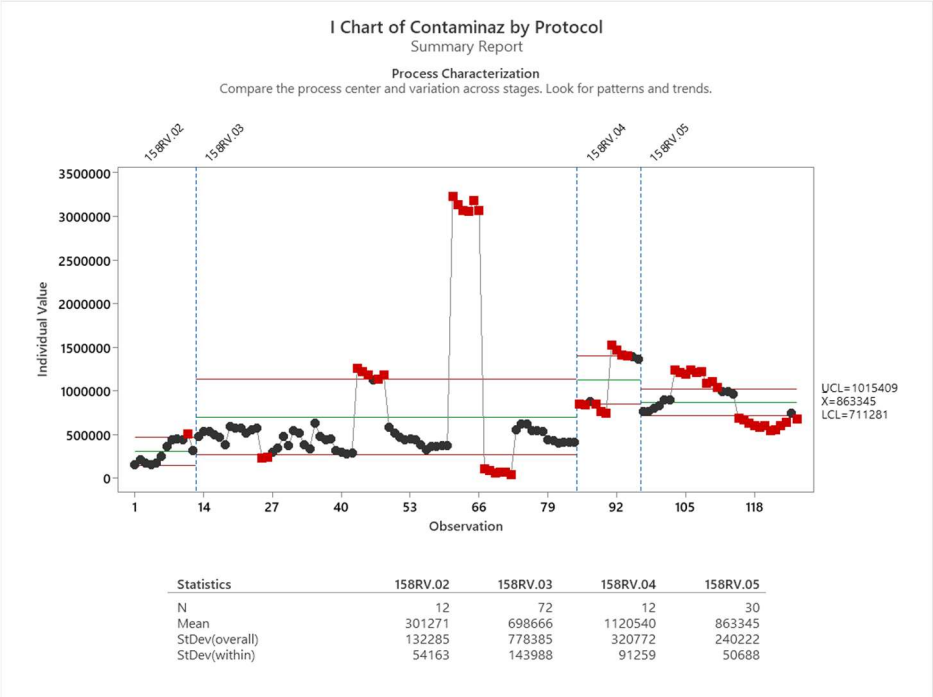


Figure 4.64 I-Chart of particulate measurements collected in the Airlock Room environment of the Via Statale facility across revisions 02, 03, 04 and 05 of the PL0158 protocol.

The average contamination level increased from 301,271 particles in Revision 02 to 698,666 particles in Revision 03, reaching the highest value in Revision 04 (1,120,540 particles). In Revision 05, the average contamination level decreased to 863,345 particles, although it remained higher than in Revisions 02 and 03. Revisions 03 and 04 exhibited the greatest process variability together with several isolated outliers, while Revision 05 showed a reduction in variability compared with Revision 04.

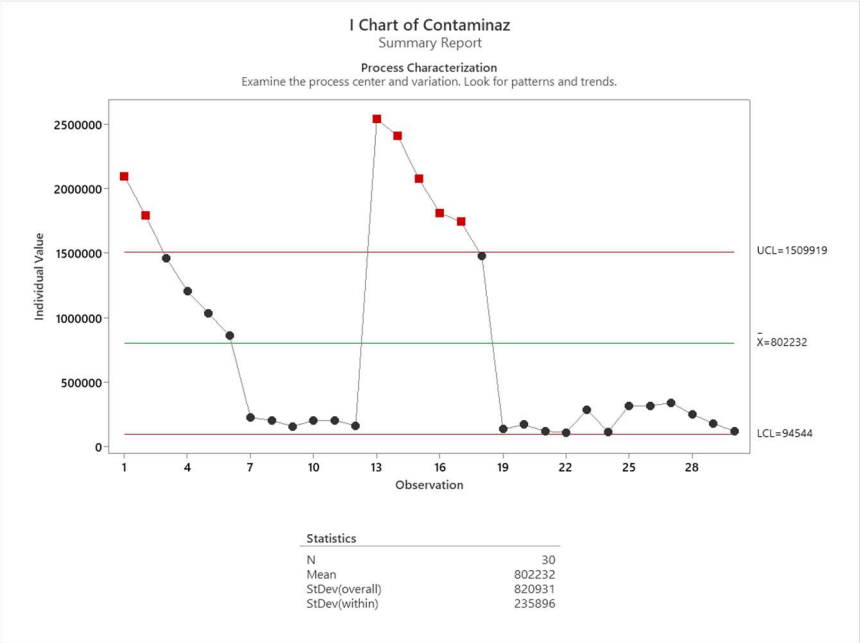


Figure 4.65 I-Chart of particulate measurements collected in the Airlock Dressing Room environment of the Via Statale facility across revision 05 of the PL0158 protocol.

The average particulate contamination was 802,232 particles, with an overall standard deviation of 820,931 particles, indicating marked process variability. Several isolated outliers exceeding the upper control limit were observed, corresponding to the highest contamination peaks recorded during the monitoring period, while the remaining measurements were characterized by substantially lower contamination levels.

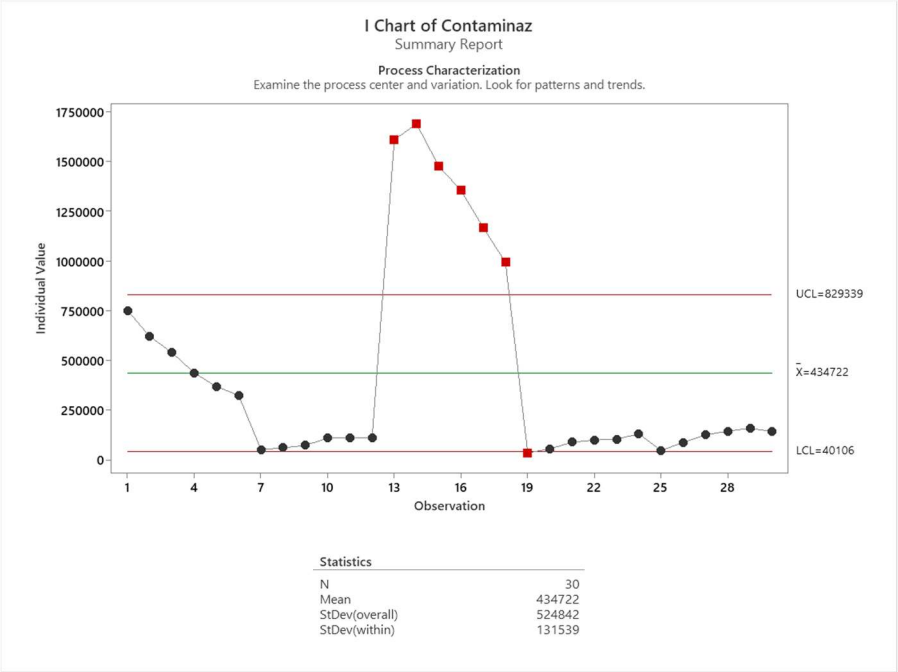


Figure 4.66 I-Chart of particulate measurements collected in the Airlock Washing Room environment of the Via Statale facility across revision 05 of the PL0158 protocol.

The average particulate contamination was 434,722 particles, with an overall standard deviation of 524,842 particles, indicating considerable process variability. Several consecutive outliers above the upper control limit were observed in the central portion of the monitoring period, corresponding to transient contamination peaks, whereas the remaining measurements were generally distributed at substantially lower contamination levels.

Finally, the same analysis was applied to the Inlet Material Room and Outlet Material Room environments. Figures 4.69 and 4.70 present the corresponding Individual Control Charts.

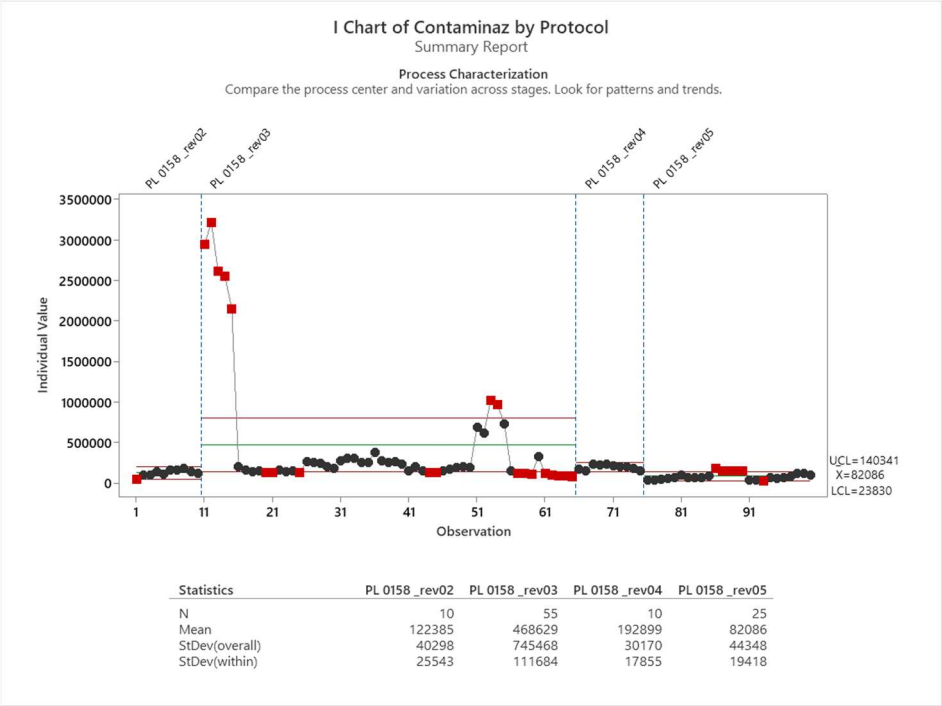


Figure 4.67 I-Chart of particulate measurements collected in the Inlet Room environment of the Via Statale facility across revision 05 of the PL0158 protocol.

Higher average contamination levels were observed during Revisions 03 (468,629 particles) and 04 (192,899 particles), together with several outliers, whereas Revision 05 showed lower contamination levels (82,086 particles) and fewer outliers.
Overall, process variability decreased progressively in the most recent revision.

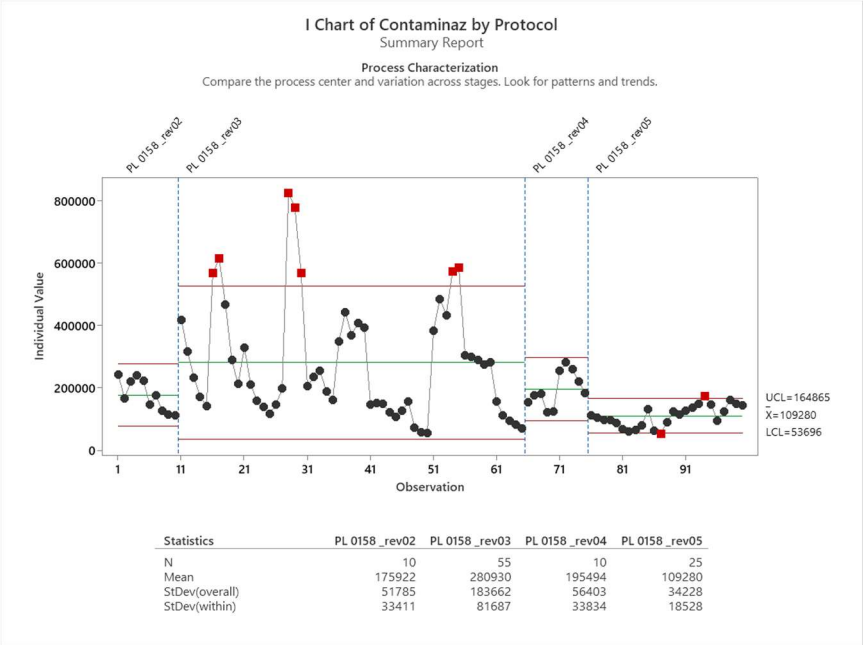


Figure 4.68 I-Chart of particulate measurements collected in the Outlet Room environment of the Via Statale facility across revision 05 of the PL0158 protocol.

The highest average contamination was observed during Revision 03 (280,930 particles), followed by a progressive decrease in Revisions 04 (195,494 particles) and 05 (109,280 particles). A limited number of outliers were identified throughout the monitoring period. Overall, the chart indicates a progressive reduction in particulate contamination levels across the investigated protocol revisions.

4.3 Process Performance and Capability Analysis

In this subsection, the performance of the monitored processes and the variability among protocol revisions were evaluated through inferential statistical analyses and process capability assessment. Since microbiological datasets were characterized by a limited number of observations and non-parametric distributions, these analyses were performed exclusively on particulate monitoring data.

One-way ANOVA tests were initially carried out to determine whether statistically significant differences were present among the different protocol revisions ($p < 0.05$ indicating that at least one group mean differed significantly). When no significant differences were identified, the entire dataset was considered as a single process and a single process capability analysis was performed. Conversely, when significant differences were detected, pairwise comparisons between consecutive revisions were carried out using two-sample t-tests ($p < 0.05$ indicating a significant difference between the two means). Process capability analyses were then performed separately for each protocol revision.

The analyses were conducted on the most representative monitored environments of the Bove and Via Statale facilities. Detailed capability analyses are presented only for the Clean Room environment, whereas the capability results obtained for the remaining monitored environments are summarized in Appendix C.

4.3.1 Via Bove facility

Table 4.7 summarizes the results of the statistical analyses performed on particulate monitoring data collected in the most representative environments of the Bove facility across the different revisions of the PL048-23 protocol. One-way ANOVA tests were used to evaluate differences among protocol revisions. When statistically significant differences were detected, pairwise comparisons were performed using two-sample t-tests. Process capability was then assessed through the Process Performance Index (Ppk), the Process Capability Index (Cpk), and the expected proportion of measurements exceeding the upper specification limit (PPM > USL).

Table 4.7 Results of ANOVA tests, t-tests, and process capability analyses performed on particulate monitoring data collected in the Bove facility across the different revisions of the PL048-23 protocol.

ID	ROOM	ISO	PL048-23 Rev.	One-Way ANOVA	2-Sample t Test	Cpk	Ppk	PPM>USL Exp. overall
14	Clean Room	7	02	0,001	/	/	0,95	2185,88
			04		/	/	1,13	338,58
			05		/	/	0,96	1949,53
13	Airlock	7	02	0,178	/	/	/	/
			04		0,101	/	0,66	23897,56
			05					
23	Manufacturing Cassette	7	02	0,277	/	/	/	/
			04		0,124	/	3,85	0
			05			/	0,86	4998,68
12	Spogliatoio donna	8	02	0,771	/	2,75	2,15	0
			04		/			
			05		/			
11	Spogliatoio uomo	8	02	0,002	/	N/D (pochi dati)		
			04		0,408	/	12,17	0
			05			/	4,41	0
15	Incoming Control room	7	02	0,847	/	8,84	6,34	0
			04		/			
			05		/			
21	Incoming Material room	7	02	0,001	/	4,59	4,83	0
			04		0,152	3,63	2,37	0
			05			/	8,29	0

To complement the statistical results reported in Table 4.7, detailed graphical analyses were performed only for the Clean Room environment, which represents the most critical production area of the Bove facility and was monitored throughout all protocol revisions.

The analysis begins with the One-way ANOVA results obtained for the Clean Room environment. Since statistically significant differences were identified among Revisions 02, 04, and 05 (Table 4.7), process capability was evaluated separately for each revision.
As shown in Figure 4.71, the one-way ANOVA revealed statistically significant differences among the three protocol revisions ($p = 0.001$). In particular, Revision 05 differed significantly from Revisions 02 and 04, whereas no statistically significant difference was observed between Revisions 02 and 04.

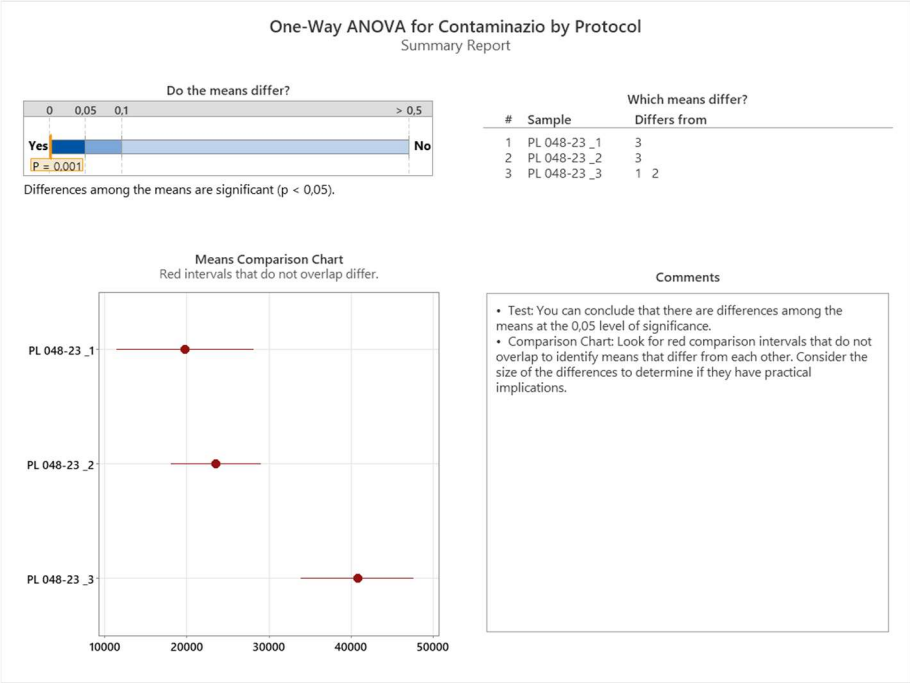


Figure 4.69 One-Way ANOVA results for particulate contamination measured in the Clean Room environment of the Bove facility across revisions 02(PL 048-23_1), 04(PL 048-23_2), and 05(PL 048-23_3) of the PL048-23 protocol.

Figure 4.72 presents the process capability analysis performed for Revision 02.

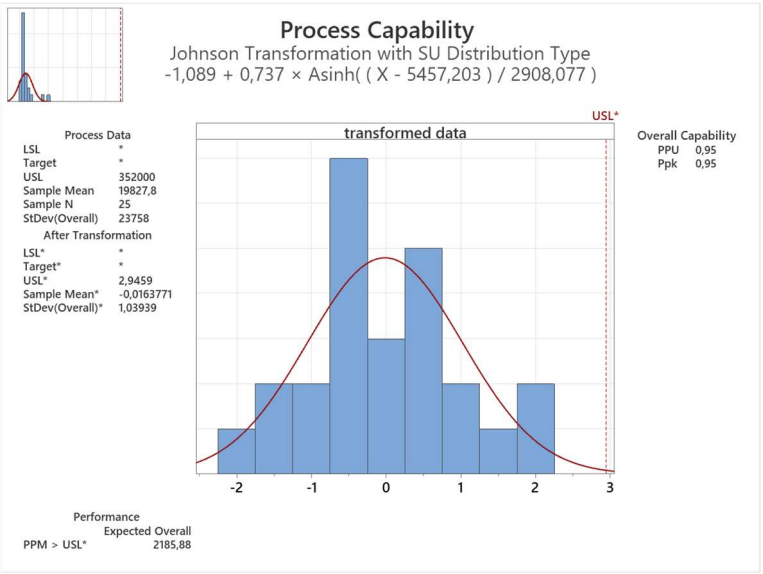


Figure 4.70 Process capability report for particulate contamination measured in the Clean Room environment of the Bove facility for revision 02 of the PL048-23 protocol

The analysis was carried out considering an upper specification limit (USL) of 352,000 particles. The monitored dataset consisted of 25 measurements, with a mean particulate contamination of 19,828 particles and an overall standard deviation of 23,758 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 0.95, corresponding to an expected 2,186 ppm of measurements exceeding the upper specification limit.

Figure 4.73 presents the process capability analysis performed for Revision 04.

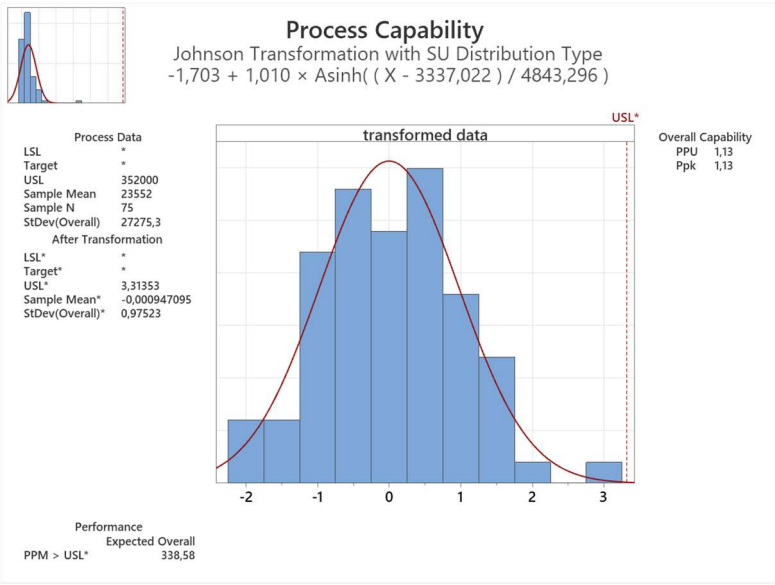


Figure 4.71 Process capability report for particulate contamination measured in the Clean Room environment of the Bove facility for revision 04

The analysis was carried out considering an upper specification limit (USL) of 352,000 particles. The monitored dataset consisted of 75 measurements, with a mean particulate contamination of 23,552 particles and an overall standard deviation of 27,275 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 1.13, corresponding to an expected 339 ppm of measurements exceeding the upper specification limit.

Figure 4.74 presents the process capability analysis performed for Revision 05.

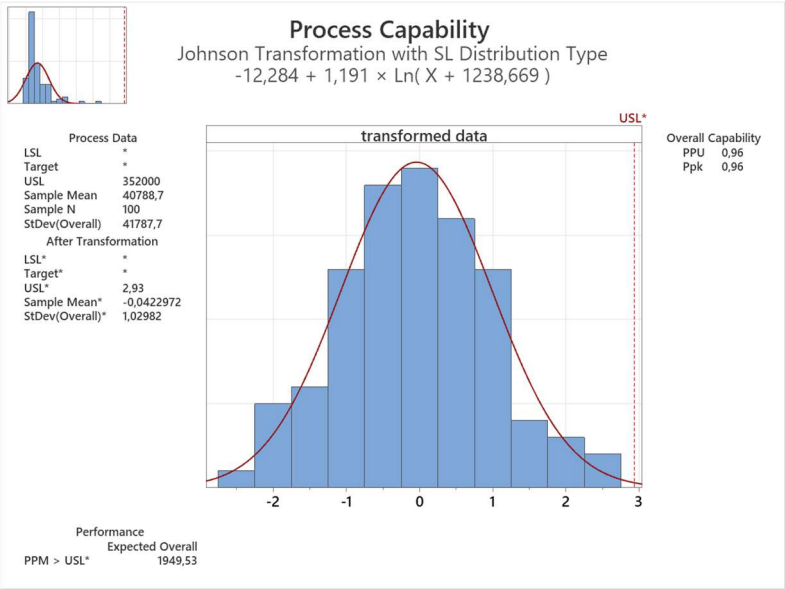


Figure 4.72 Process capability report for particulate contamination measured in the Clean Room environment of the Bove facility for revision 05

The analysis was carried out considering an upper specification limit (USL) of 352,000 particles. The monitored dataset consisted of 100 measurements, with a mean particulate contamination of 40,789 particles and an overall standard deviation of 41,788 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 0.96, corresponding to an expected 1,950 ppm of measurements exceeding the upper specification limit.

Overall, Revision 04 exhibited the highest process capability (Ppk = 1.13), corresponding to the lowest expected proportion of non-conforming measurements (339 ppm). In contrast, Revisions 02 and 05 showed comparable process performance (Ppk = 0.95 and 0.96, respectively), with expected non-conforming rates of 2,186 ppm and 1,950 ppm.

4.3.2 Via Statale facility

Table 4.8 summarizes the results of the statistical analyses performed on particulate monitoring data collected in the most representative environments of the Statale facility across the different revisions of the PL0158 protocol. One-way ANOVA tests were used to evaluate differences among protocol revisions. Whenever statistically significant differences were detected, pairwise comparisons were performed using two-sample t-tests. Process capability was subsequently evaluated using the Process Performance Index (Ppk), the Process Capability Index (Cpk), and the expected proportion of measurements exceeding the upper specification limit (PPM > USL).

Table 4.8 Results of ANOVA tests, t-tests, and process capability analyses performed on particulate monitoring data collected in the Via Statale facility across the different revisions of the PL0158 protocol.

ID	ROOM	ISO	PL0158 Rev.	One-Way ANOVA	2-Sample t Test	Cpk	Ppk	PPM>USL Exp. overall
1	Clean room	7	05	/	/	/	1,43	9,52
		8	02	0,001	0,001	/	46	0
			03		0,003	/	1,07	681,65
			04		0,023	/	1,17	213,65
			05			/	28,42	0
2	Inlet Materials room	8	02	0,001	0,001	/	/	/
			03		0,009	/	0,67	22994
			04		0,001	/	/	/
			05			/	28,61	0
3	Outlet Materials room	8	02	0,001	0,001	13,53	7,8	0
			03		0,007	/	4,7	0
			04		0,001	12,18	7,47	0
			05			47,14	33,22	0
			02	0,001	0,001	/	13,58	0

7	Airlock	8	03			/	0,66	23349,68
					0,003			
			04			/	5,64	0
					0,023			
			05			/	6,47	0
5	Airlock Dressing room	8	05	/	/	/	1,45	0
4	Airlock Washing room	8	05	/	/	/	1,91	0

To complement the statistical results reported in Table 4.8, detailed graphical analyses were performed only for the Clean Room environment, which represents the most critical production area of the Via Statale facility. Since the Clean Room was reclassified from ISO 8 to ISO 7 in Revision 05 of the PL0158 protocol, separate process capability analyses are presented for the two ISO classifications.

Figure 4.75 presents the results of the one-way ANOVA performed on particulate contamination measured in the ISO 8 Clean Room environment across Revisions 02, 03, 04, and 05 of the PL0158 protocol.

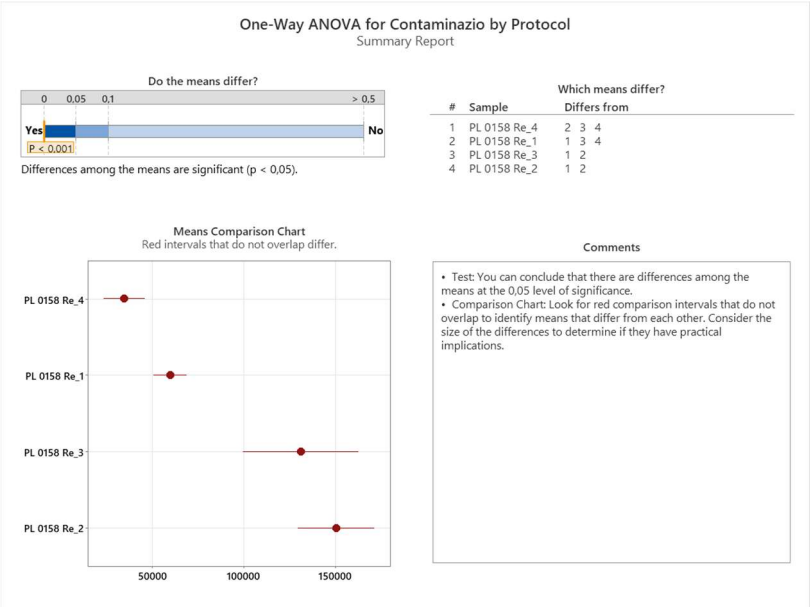


Figure 4.73 One-Way ANOVA results for particulate contamination measured in the ISO 8 Clean Room environment of the Via Statale facility across revisions 02(RE_1), 03(RE_2), 04(RE_3) and 05(RE_4) of the PL0158 protocol.

As shown in Figure 4.75, the one-way ANOVA revealed statistically significant differences among the four protocol revisions ($p < 0.001$).

The pairwise two-sample t-tests revealed that Revision 05 differed significantly from Revisions 02, 03, and 04, whereas Revision 02 also differed significantly from Revisions 03 and 04. No statistically significant difference was observed between Revisions 03 and 04, indicating comparable mean particulate contamination levels during these monitoring periods. The corresponding p-values are reported in Table 4.8.

Figure 4.76 presents the process capability analysis performed for Revision 02 of the ISO 8 Clean Room.

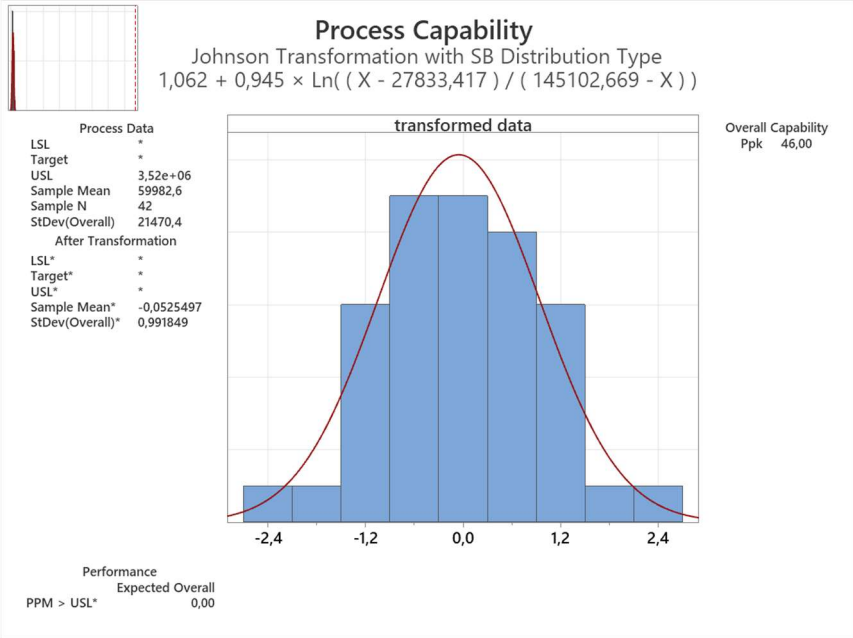


Figure 4.74 Process capability report for particulate contamination measured in the ISO 8 Clean Room environment of the Via Statale facility for revision 02

The analysis was performed considering an upper specification limit (USL) of 3,520,000 particles. The monitored dataset consisted of 42 measurements, with a mean particulate contamination of 59,983 particles and an overall standard deviation of 21,470 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 46.00, corresponding to an expected 0 ppm of measurements exceeding the upper specification limit, indicating excellent process capability with respect to the specified contamination limit.

Figure 4.77 presents the process capability analysis performed for Revision 04.

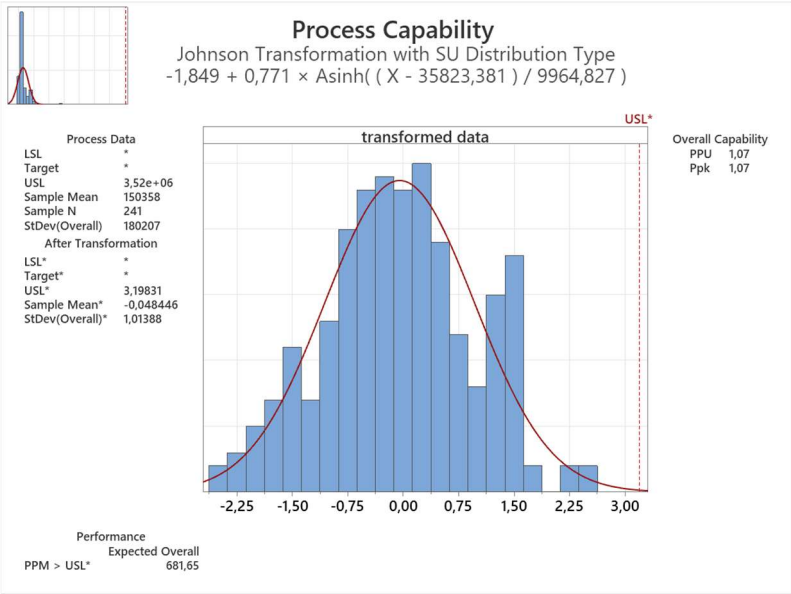


Figure 4.75 Process capability report for particulate contamination measured in the ISO 8 Clean Room environment of the Via Statale facility for revision 03

The analysis was performed considering an upper specification limit (USL) of 3,520,000 particles. The monitored dataset consisted of 241 measurements, with a mean particulate contamination of 150,358 particles and an overall standard deviation of 180,207 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 1.07, corresponding to an expected 682 ppm of measurements exceeding the upper specification limit. The process therefore exhibited acceptable capability, although lower than that observed during Revision 02.

Figure 4.78 presents the process capability analysis performed for Revision 04.

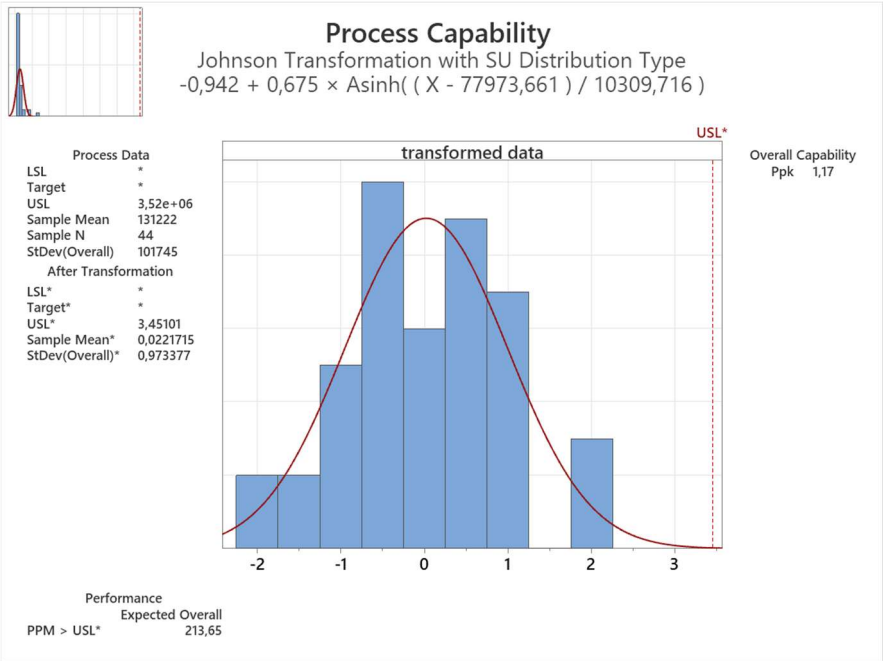


Figure 4.76 Process capability report for particulate contamination measured in the ISO 8 Clean Room environment of the Via Statale facility for revision 04

The analysis was performed considering an upper specification limit (USL) of 3,520,000 particles. The monitored dataset consisted of 44 measurements, with a mean particulate contamination of 131,222 particles and an overall standard deviation of 101,745 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 1.17, corresponding to an expected 214 ppm of measurements exceeding the upper specification limit. Compared with Revision 03, the process capability slightly improved, indicating a lower expected proportion of measurements exceeding the specification limit.

Figure 4.79 presents the process capability analysis performed for Revision 05.

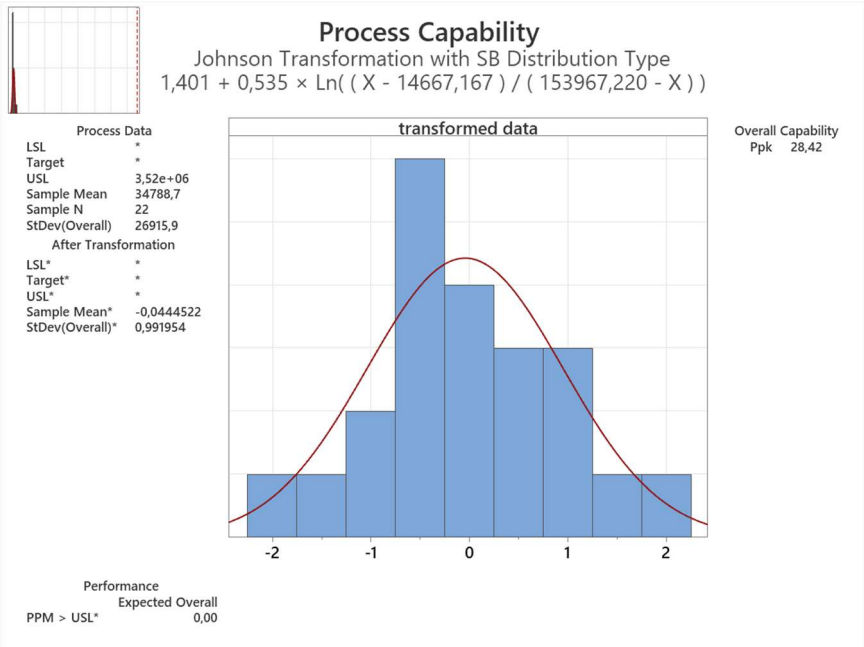


Figure 4.77 Process capability report for particulate contamination measured in the ISO 8 Clean Room environment of the Via Statale facility for revision 05.

The analysis was performed considering an upper specification limit (USL) of 3,520,000 particles. The monitored dataset consisted of 22 measurements, with a mean particulate contamination of 34,789 particles and an overall standard deviation of 26,916 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 28.42, corresponding to an expected 0 ppm of measurements exceeding the upper specification limit. This result indicates an excellent process capability, with all observations remaining well below the specified acceptance limit.

The final analysis refers to the ISO 7 Clean Room environment. Since this classification was introduced only in Revision 05 of the PL0158 protocol, no comparison among protocol revisions could be performed. Therefore, only the process capability analysis for Revision 05 is presented.

Figure 4.80 presents the process capability analysis performed on particulate contamination data collected in the ISO 7 Clean Room environment of the Via Statale facility during Revision 05 of the PL0158 protocol.

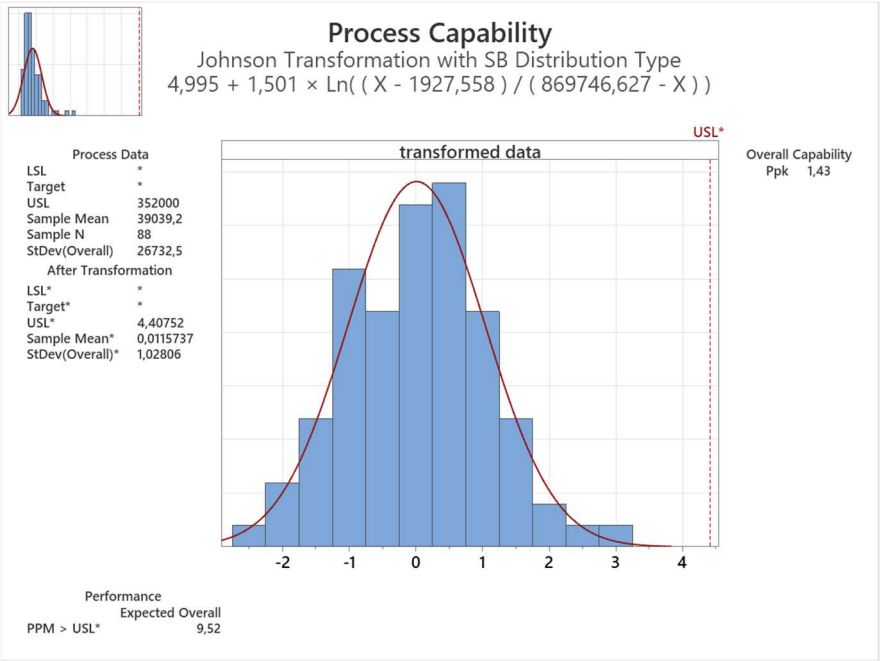


Figure 4.78 Process capability report for particulate contamination measured in the ISO 7 Clean Room environment of the Via Statale facility for Revision 05 of the PL0158 protocol.

The analysis was carried out considering an upper specification limit (USL) of 352,000 particles. The monitored dataset consisted of 88 measurements, with a mean particulate contamination of 39,039 particles and an overall standard deviation of 26,733 particles. Following Johnson transformation, the process capability analysis yielded a Ppk value of 1.43, corresponding to an expected 9.52 ppm of measurements exceeding the upper specification limit.

The process capability analysis yielded a Ppk value of 1.43, corresponding to an expected 9.52 ppm of measurements exceeding the upper specification limit. These results indicate a capable process, with particulate contamination consistently remaining within the required acceptance criteria throughout the monitoring period.

5 Discussion

5.1 Process Variability

5.1.1 Via Bove Facility

The analyses performed on environmental monitoring data from the via Bove facility highlighted different variability behaviors among the monitored controlled environments and across the different revisions of the PL048-23 protocol. The results obtained from particulate, surface microbiological, and airborne microbiological monitoring showed generally coherent trends, allowing the observed variations to be correlated with the modifications progressively introduced throughout the protocol revisions, including the introduction of new equipment, the increase in production activities, the higher number of operators, and the progressive modification of personnel and material flows within the classified areas.

Among all the analyzed environments, the Clean Room (room 14) represented the most critical operational area and the environment most directly involved in production activities.

Particulate monitoring analyses highlighted a progressive increase in both mean values and process variability from Rev.02 to Rev.05, together with the presence of several out-of-control points in the I-Charts. This behavior may be associated with the progressive increase in production activities, the higher number of operators present in the environment, and the introduction of additional equipment within the area. Since the Clean Room represented the largest and most frequently occupied environment within the facility, these conditions may also explain the elevated number of outliers observed during the latest revisions.

Surface microbiological monitoring within the Clean Room showed a decrease in process mean values from Rev.02 to Rev.04, followed by a slight increase during Rev.05. At the same time, variability decreased during Rev.04 and subsequently increased again during Rev.05, with the presence of several out-of-control points in the Individual Chart.

Airborne microbiological monitoring showed a similar trend, with an initial reduction in microbiological mean values followed by an increase during Rev.05, together with greater data dispersion. The agreement between particulate and microbiological monitoring suggests that the increase in operational activities and personnel presence influenced the environmental conditions within the main production area.

The Manufacturing cassette environment, dedicated to the production of specific medical devices intended for radiopharmaceutical drug preparation, showed a partially different behavior. During Rev.04, additional equipment was introduced within the environment without showing an immediate negative impact on particulate contamination trends. Rev.05 mainly included updates to the environmental monitoring procedure, such as layout revisions, updates of contamination limits and sampling locations, modifications of environmental specifications, and the introduction of limits for operators' gloves. These

changes did not appear to have a significant direct impact on particulate contamination levels. The increase in mean values and process variability observed during Rev.05 is therefore more likely related to the progressive use of the equipment introduced in the previous revision and to the increase in routine production activities performed within the environment.
Considering the limited number of observations available for Rev.02, the comparison between Rev.04 and Rev.05 provides a more representative assessment of the process trend.
Surface microbiological monitoring showed relatively stable contamination levels and variability across the two revisions, suggesting that operator-related contamination remained under control. This behaviour is consistent with the strict personnel management procedures adopted within the clean room, including controlled access, specific training requirements and the mandatory use of personal protective equipment such as gloves and masks.
Airborne microbiological monitoring showed a reduction in contamination levels during Rev.05, indicating an improvement in environmental conditions. This trend is likely associated with the effectiveness of the HVAC system in maintaining adequate air quality and microbiological control within the monitored environment.

The Airlock environment showed intermediate variability conditions compared to the strictly operational production environments, since the Airlock represents the transition area between the ISO 8 changing rooms and the ISO 7 Clean Room. Personnel flow play a critical role in maintaining environmental balance between these classified areas by adopting specific washing and sanitization measurement before accessing the manufacturing Clean Room.
Particulate monitoring analyses highlighted an increase in both process mean values and variability between Rev.02 and Rev.04, associated with the progressive increase in production activities and, consequently, personnel flow within the environment. Subsequently, during Rev.05, the process appeared to return to more controlled conditions, suggesting a progressive standardization of operational activities and personnel behavior.
Surface microbiological monitoring showed a progressive improvement in control conditions, highlighting a significant reduction in microbiological mean values together with narrower control limits throughout the successive protocol revisions. Similarly, airborne microbiological monitoring also showed a reduction in both processes mean values and variability between Rev.04 and Rev.05.
Overall, these results indicate a progressive improvement in environmental management and increased personnel awareness regarding appropriate behavior within classified areas.

The Incoming Control Room, dedicated to inspection and verification activities for incoming components intended for production and storage, showed an increase in both process mean values and variability between Rev.02 and Rev.04 in particulate monitoring analyses. This behavior may be associated with increased personnel flow and the introduction of additional operational equipment. Subsequently, during Rev.05, the process appeared more stable and controlled.

Surface microbiological monitoring also showed a progressive reduction in both microbiological mean values and variability from Rev.02 to Rev.05. Since access to the Incoming Control Room is restricted to specifically trained personnel, operator-related contamination is effectively controlled, contributing to the maintenance of stable environmental conditions.
Similarly, airborne microbiological monitoring highlighted an improvement in environmental conditions between Rev.04 and Rev.05, with a slight reduction in microbiological mean values and overall good process stability.
A similar behavior was observed in the Incoming Material Room, dedicated to the transfer of materials from the internal warehouse toward production activities. Particulate monitoring analyses highlighted increased variability during Rev.04, followed by a return to more stable conditions during Rev.05. At the same time, surface microbiological monitoring showed a progressive reduction in both process mean values and microbiological variability throughout the different protocol revisions.
Airborne microbiological monitoring also highlighted a progressive reduction in both process mean values and variability from Rev.02 to Rev.05. The restricted access policy and the requirement for specific operator training likely contributed to maintaining stable environmental parameters and reducing microbiological contamination. These results indicate that the progressive standardization of operational activities, together with effective personnel and material flow management, contributed to the overall improvement of environmental conditions observed during the latest revisions.

Regarding the changing rooms, different behaviors were observed between the women's and men's environments.
In the Women's Changing Room, particulate monitoring highlighted increased variability associated with the increase in production activities and the higher number of operators accessing the environment. Since this changing room is part of the routine personnel flow toward the clean room, the observed behaviour is consistent with the greater operational activity recorded during the most recent protocol revisions. Surface microbiological monitoring also showed relatively high variability values, with several out-of-control points particularly during Rev.04 and Rev.05.
However, airborne microbiological monitoring highlighted a reduction in both process mean values and variability from Rev.04 to Rev.05, suggesting improved environmental control and effective management of personnel flows.
In the Men's Changing Room, particulate monitoring showed relatively similar variability conditions between Rev.04 and Rev.05, with a slight reduction in mean values during the latest revision. Airborne microbiological monitoring also highlighted a reduction in both process mean values and variability during Rev.05 compared to Rev.04. Surface microbiological monitoring instead showed increased variability during Rev.04 followed by a subsequent reduction during Rev.05.

Unlike the Women's Changing Room, the Men's Changing Room is not part of the routine production flow and is mainly used by visitors and maintenance personnel. The more sporadic and less standardized use of the environment may therefore contribute to the variability observed in some monitoring results. Overall, both environments remained under control, while the latest revisions highlighted improvements in environmental management and monitoring performance.

Overall, the analyses performed highlighted that the environments most directly involved in production activities showed higher variability levels compared to environments dedicated to specific supporting functions, such as the Incoming Control Room, Incoming Material Room, Airlock and Pass Area. The variations observed across the different protocol revisions were closely associated with changes in production activities, personnel and material flows, as well as with the introduction and routine use of additional equipment within the controlled environments.

In particular, the main production environments, such as the Clean Room and Manufacturing Cassette area, showed the highest variability levels together with the greatest number of out-of-control points. Conversely, environments mainly dedicated to material transfer, inspection activities and personnel transit generally exhibited more stable behaviour throughout the latest protocol revisions.

In several monitored environments, the most recent revisions were characterized by reduced variability and narrower control limits, suggesting improved process standardization, greater operator awareness and more effective management of personnel and material flows within the classified areas.

Overall, the obtained results indicate that the environmental control system adopted within the Bove facility was effective in maintaining contamination levels under control, as demonstrated by the general compliance of the monitored environments and the progressive stabilization observed in several areas during the latest protocol revisions

5.1.2 Via Statale Facility

The analyses conducted on the environmental monitoring data of the Via Statale facility highlighted a progressive evolution of environmental control conditions throughout the different revisions of protocol PL0158.

The successive protocol revisions reflected several changes introduced within the facility. Rev.02 included the addition of particulate and microbiological monitoring associated with pressure test activities. Rev.03 introduced an additional sampling point following modifications to the clean room layout. Rev.04 expanded the monitoring program through the introduction of Laminar Flow Hood controls, temperature and relative humidity monitoring, and the definition of the maximum number of operators allowed within each area. Finally, Rev.05 included a revised environmental monitoring plan, revalidation activities, the introduction of the Airlock Dressing area and the implementation of a new HVAC system.

Overall, Rev.03 and Rev.04 represented the phases characterized by the highest environmental variability, whereas Rev.05 showed, in most monitored environments, a progressive reduction in variability and improved process stability. This behaviour may be associated with the combined effect of increased process standardization and the replacement of the previous HVAC system.
The Airlock represents the personnel access area to the clean room and plays a key role in preventing contamination transfer between non-classified and classified environments. From a particulate monitoring perspective, the analyses highlighted a marked increase in both process mean and variability during Rev.03 and especially Rev.04, followed by a reduction in Rev.05. The presence of several outlier points during the intermediate revisions suggests a phase of increased instability, likely associated with changes in personnel flow patterns and modifications to the environmental layout. The improved behaviour observed during Rev.05 may also reflect the positive impact of the new HVAC system on environmental control conditions.

Surface microbiological monitoring, on the other hand, showed generally less variability. Excluding Rev.02 due to the limited number of available observations, Rev.03 showed the highest microbiological variability, while Rev.04 appeared to be the most stable revision. Rev.05 displayed a slight increase in data dispersion, although without significant outlier . Airborne microbiological monitoring also confirmed a progressive improvement of environmental conditions: considering only the statistically reliable revisions, Rev.05 showed reduced variability compared to Rev.03 and no outlier points, suggesting improved environmental control conditions. This behaviour may be associated with both the progressive standardization of personnel activities and the implementation of the new HVAC system introduced during Rev.05.
The Airlock Dressing Room and Airlock Washing Room environments, introduced in the most recent revision of the protocol, showed generally stable conditions both in terms of surface and airborne microbiological monitoring. These environments were created following the reorganization of the previous Airlock area, which originally consisted of a single room. The new layout separates hand-washing activities from gowning procedures, improving personnel flow management and reducing the risk of cross-contamination during access to the clean room. In both environments, some variability oscillations were observed during the initial monitoring phases, likely associated with the implementation of the new operational procedures and the adaptation of personnel to the revised workflow.
However, all observations remained within control limits and no evident instability patterns were identified, suggesting rapid standardization of the activities performed within these environments.
The Clean Room represents the main production environment of the facility and progressively evolved both from an organizational and environmental classification perspective, transitioning from ISO 8 in the initial revisions to ISO 7 in the most recent protocol revision.

From the particulate monitoring perspective, the initial revisions of the Clean Room under ISO 8 classification showed conditions of greater instability, particularly during Rev.03, characterized by high mean values, wide data dispersion and several outliers. This behaviour may be associated with clean room layout modifications, the introduction of additional sampling points and increased operational activities resulting from the progressive adaptation of the facility and its production processes.
In the subsequent revisions, a progressive reduction in both process mean values and variability was observed, suggesting improved process stability and increasing standardization of operational activities. This trend culminated in Rev.05, when the introduction of the new HVAC system coincided with the formal reclassification of the Clean Room from ISO 8 to ISO 7.
Although environmental monitoring data collected during the previous years already demonstrated compliance with ISO 7 particulate limits, Rev.05 was characterized by lower variability and greater stability of environmental parameters across the monitored area.
These results suggest that the combined effect of improved environmental control systems, process standardization and optimized personnel and material flow management contributed to the achievement of more stable operating conditions within the main production environment.

Surface microbiological monitoring also confirmed this evolutionary trend. During the revisions associated with the ISO 8 classification, the process appeared overall stable but characterized by the presence of several isolated outlier points, particularly during the intermediate revisions. Rev.04 appeared to be the most stable phase, whereas Rev.05 showed slightly wider oscillations, probably associated with increased operational activities and personnel flows during the environmental classification transition phase.
Airborne microbiological monitoring showed a similar behavior. The intermediate revisions displayed higher variability levels and a greater statistical uncertainty, particularly during Rev.03 and Rev.04, whereas Rev.05 showed reduced average contamination levels and greater process stability compared to the previous revisions. The transition of the Clean Room from ISO 8 to ISO 7 coincided with the introduction of the new HVAC system, whose improved air treatment performance likely contributed to the observed reduction in contamination levels and environmental fluctuations

The Inlet Material Room and Outlet Material Room showed similar behaviors, as both are strictly related to the incoming and outgoing material flows involved in the production process. Particulate monitoring in both environments showed particularly high mean values and variability during Rev.03, accompanied by several out-of-control points, whereas the subsequent revisions displayed a progressive reduction in data dispersion and improved process stability. These findings suggest that the initial implementation phases of the protocol and the new operational workflows initially increased environmental variability, which was subsequently reduced through process standardization.
From the surface microbiological perspective, the Inlet Material Room showed a reduction in both process mean and variability during Rev.05 compared to Rev.04, suggesting a progressive

improvement in incoming material management. Conversely, the Outlet Material Room showed an increase in both mean values and data dispersion during Rev.05, probably associated with increased operational activities and outgoing material flows.
Regarding airborne microbiological monitoring, both environments showed overall stable conditions without evident out-of-control signals. The differences observed among revisions remained limited and suggest generally adequate microbiological control of material flows within the facility.
Overall, the integrated environmental monitoring analysis of the Via Statale facility highlights that the main criticalities were observed during the intermediate revisions of the protocol, concurrently with layout modifications, introduction of new environments, and increased operational and monitoring activities.
The most recent revisions, particularly Rev.05, instead show a progressive reduction in variability and a general improvement in environmental stability, both from the particulate and microbiological perspectives. These results suggest consolidation of the environmental control system and increasing standardization of operational procedures within the classified environments of the Via Statale facility.
Despite the variability observed in some production environments, all monitored areas remained within the applicable particulate and microbiological contamination limits throughout the study period.

5.2 Process Capability

From a Quality Assurance perspective, process capability represents a valuable preventive tool, as it allows the evaluation of how effectively the combination of environmental control systems, operational procedures, and personnel behaviors can maintain contamination levels within the established specification limits. Beyond assessing current process performance, capability analysis provides information about the available operating margin and the robustness of the monitored environments with respect to future variations.
In this sense, capability indices support the identification of environments that, although currently compliant, may be more susceptible to future deviations caused by increased process variability, changes in production activities, or deterioration of environmental control conditions. Consequently, capability analysis can support preventive decision-making activities, such as anticipating HVAC maintenance interventions, improving operational procedures, increasing monitoring frequency, or implementing corrective actions before actual non-conformities occur.
Overall, the capability results provide complementary information to the variability analyses, allowing the evaluation of both the current robustness of the monitored processes and their potential evolution over time to be evaluated.

5.2.1 *Via Bove facility*

The capability analysis performed on the Via Bove facility revealed heterogeneous behaviour among the monitored environments.

Areas characterized by limited operational activity, restricted personnel access, and the presence of specifically trained operators generally exhibited high capability values. In particular, the Incoming Control Room and Incoming Material Room showed Ppk values ranging from 2.37 to 8.29, indicating highly capable environmental conditions and a robust ability to maintain particulate contamination levels within the established limits. The relatively stable environmental conditions observed throughout the protocol revisions were therefore reflected in high capability indices.

Conversely, environments directly involved in production activities, such as the Clean Room and Manufacturing Cassette Area, generally exhibited lower capability values. The Clean Room showed Ppk values ranging between 0.95 and 1.13 across the analyzed revisions, while the Manufacturing Cassette Area exhibited values between 0.86 and 3.85 depending on the protocol revision. Although these environments remained compliant with the applicable contamination limits, the increased variability associated with production activities, operator presence, and the introduction of additional equipment reduced the gap between the environmental contamination distribution and the specification limits.

A similar behaviour was observed in the Airlock and changing room environments. While these areas remained under control, their capability values were influenced by personnel flow and gowning activities, which represent important sources of particulate generation. Nevertheless, the latest protocol revisions generally showed improved capability compared with intermediate revisions, suggesting that the progressive achievement of stable and continuous production conditions, together with increased process standardization and operator awareness, contributed to reducing environmental variability over time.

Overall, the capability assessment indicates that the Via Bove facility maintains adequate environmental control conditions. However, production-related environments represent the most critical areas and therefore require continued monitoring in order to preserve sufficient operating margins with respect to the specification limits.

In particular, the Clean Room and Manufacturing Cassette Area deserve special attention during future monitoring activities, as they showed the lowest capability values among the monitored environments (Ppk values ranging from 0.86 to 1.13), indicating reduced operating margins with respect to the specification limits despite continued compliance with environmental requirements.

5.2.2 *Via Statale facility*

Compared with the Via Bove facility, the Via Statale site generally demonstrated higher and more homogeneous capability levels across the monitored environments. Most environments exhibited Ppk values greater than 1.33 and several areas showed values substantially above 2.0, indicating highly capable operating conditions and large operating margins with respect to the

established contamination limits. These results suggest that particulate contamination levels remained consistently far from the specification limits and that the probability of future non-conformities was consequently very low.

The Airlock Dressing Room and Airlock Washing Room represented examples of capable processes, with Ppk values of 1.45 and 1.91, respectively. Despite being transition areas characterized by personnel movement, the capability analyses highlighted excellent environmental performance, confirming the effectiveness of the operational procedures adopted and the improved performance of the new HVAC system introduced during the latest validation protocol revision.

The Inlet Material Room and Outlet Material Room also showed satisfactory capability values. Although the Inlet Material Room exhibited a lower Ppk value during Rev.03 (0.67), capability improved substantially in the latest validation protocol revision, reaching a Ppk value of 28.61. Similarly, the Outlet Material Room maintained high capability throughout the study period, with Ppk values ranging between 4.7 and 33.22. These results reflect the progressive stabilization of material flows and the effectiveness of the procedural improvements introduced throughout the successive validation protocol revisions. In these environments, layout modifications, revised monitoring plans, and increased procedural standardization appear to have positively contributed to process performance.

Particular attention should be dedicated to the Clean Room environment, which underwent a transition from ISO 8 to ISO 7 classification during the study period. During the ISO 8 phase, capability values remained relatively modest, with Ppk values of 1.07 and 1.17 observed in Rev.03 and Rev.04 of the validation protocol, respectively. Following the introduction of the new HVAC system and the reclassification to ISO 7 in Rev.05, process capability improved markedly, reaching a Ppk value of 28.42. Although temporary increases in variability were observed during the intermediate validation protocol revisions, the capability analyses confirmed that the environmental control measures implemented during the requalification process were effective in maintaining contamination levels within the required limits. The substantial improvement observed in the latest revision further supports the effectiveness of the corrective and preventive actions introduced throughout the validation protocol evolution.

The Airlock environment showed a more variable behaviour across the different validation protocol revisions. While a lower capability value was observed during Rev.03 (Ppk = 0.66), subsequent revisions showed a marked improvement, reaching Ppk values of 5.64 and 6.47 in Rev.04 and Rev.05, respectively. These results suggest that the modifications introduced to the environmental control system, together with increasing process standardization, contributed significantly to improving environmental robustness.

Overall, the Via Statale facility demonstrated a high level of process capability, with most monitored environments operating with substantial safety margins relative to the specification limits. These findings confirm the effectiveness of the environmental monitoring strategy, the benefits associated with the new HVAC system and Clean Room reclassification, and the progressive improvement of process control achieved through successive validation protocol revisions.

6 Conclusions

This thesis work was carried out during a curricular internship at SIDAM S.r.l. within the Quality Assurance department and focused on the collection, organization, and statistical analysis of environmental monitoring and microbiological control data generated within the company's production facilities.
One of the most significant aspects of the project involved the collection and structuring of historical data originating from different facilities, monitoring protocols, and successive protocol revisions. Before performing the statistical analyses, a substantial effort was required to organize, verify, and standardize the available information in order to build consistent databases suitable for further processing. This activity resulted in the creation of a structured data repository that may also support future Quality Assurance activities.
Once organized, the data were analyzed using Minitab software through the application of descriptive and inferential statistical methods. In particular, distribution analyses, statistical hypothesis tests (ANOVA and t-tests), control charts, and process capability analyses were performed. These tools allowed objective evaluation of process behavior over time, identification of differences among protocol revisions, and detection of areas characterized by increased variability.
The results showed that the monitored environments generally maintained contamination levels within the limits required by their respective environmental classifications. Despite the variability observed in some production-related environments, all monitored areas remained compliant with the applicable particulate and microbiological contamination limits throughout the study period.

The analyses also demonstrated that environments directly involved in production activities tend to exhibit greater variability than support and transition areas, mainly due to operational activities, personnel flow, equipment installation, and increased production volumes. In several cases, improved environmental performance was observed in the most recent protocol revisions, suggesting progressive process standardization and increasing maturity of the environmental control system.

Process capability analyses confirmed that most monitored environments maintained adequate operating margins with respect to specification limits. In addition to verifying process compliance, these analyses allowed identification of production-related environments that are more sensitive to variability and may require particular attention during future monitoring activities. In this respect, process capability proved to be a valuable tool not only for assessing current performance but also for supporting preventive actions aimed at avoiding future non-conformities.

Several limitations were encountered during the project. The main challenges were related to the heterogeneous nature of the available data, the presence of multiple protocol revisions, and the limited number of observations available for some monitoring periods. Furthermore, some historical reports contained missing measurements, incomplete information, or values that were difficult to interpret, requiring additional verification during the database construction phase. Although these aspects did not compromise the overall validity of the study, they highlight the importance of standardized procedures for data recording and management.

The activities performed during this project represent an important starting point for future developments. First, the database created during the study can be continuously updated with new environmental monitoring results, progressively expanding the available historical records and improving the traceability and accessibility of information within the Quality Assurance department. The structured organization of the data provides a centralized repository that can support routine monitoring activities and facilitate the rapid identification of environmental trends over time.

Furthermore, the statistical methodology developed during this work introduced an objective approach for evaluating environmental monitoring data, allowing the identification of environments characterized by higher variability and potentially greater contamination risk. In this perspective, the developed framework may support future risk-based monitoring strategies, helping the company optimize monitoring activities and focus attention on areas requiring greater control.

The database and analytical approach could also be extended to other types of quality-related information managed within the company, such as non-conformities, deviations, complaints, and product quality controls. Future integration of these datasets may provide a more comprehensive understanding of the relationships between environmental conditions and product quality, supporting data-driven decision-making and continuous improvement initiatives.

Another possible development involves the implementation of automated systems for data collection, database updating, and graphical visualization of monitoring results, reducing the manual effort required for information management while improving the accessibility of environmental performance indicators.
An example of the database structure and of the graphical visualization tools developed during this project is reported in Figures 6.1-6.2-6.3. The system allows environmental monitoring data from different facilities, environments, and protocol revisions to be stored in a centralized repository and visualized through interactive dashboards. These functionalities facilitate trend analysis, comparison of historical monitoring results, and support Quality Assurance decision-making activities.

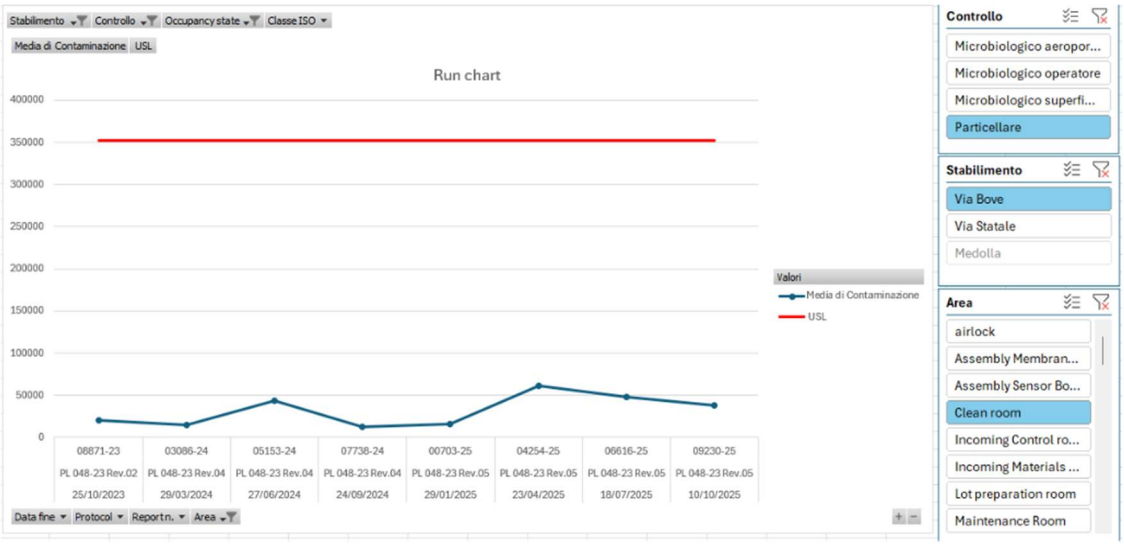


Figure 6.1 Interactive dashboard developed for the visualization and comparison of environmental monitoring data from different company facilities.

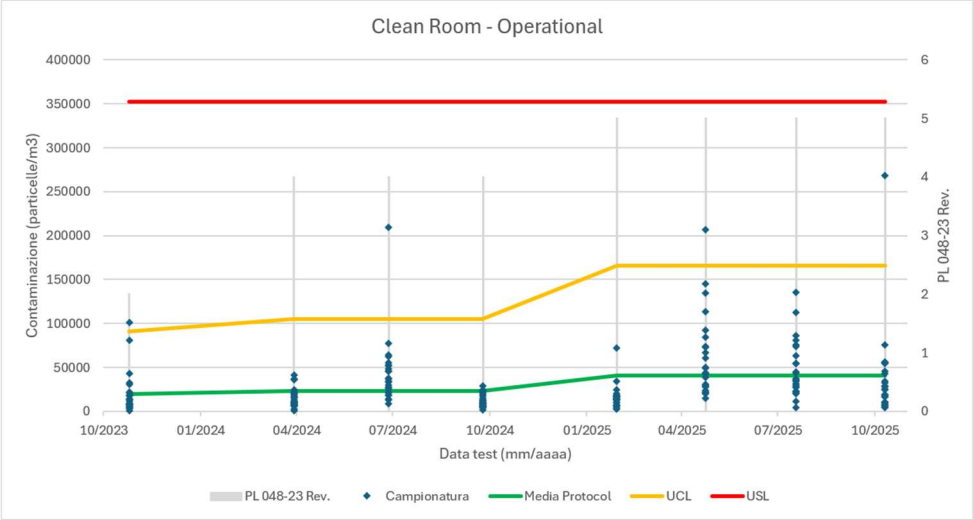


Figure 6.2 Example of run chart visualization showing historical monitoring results, individual sampling values, and protocol revision progression over time.

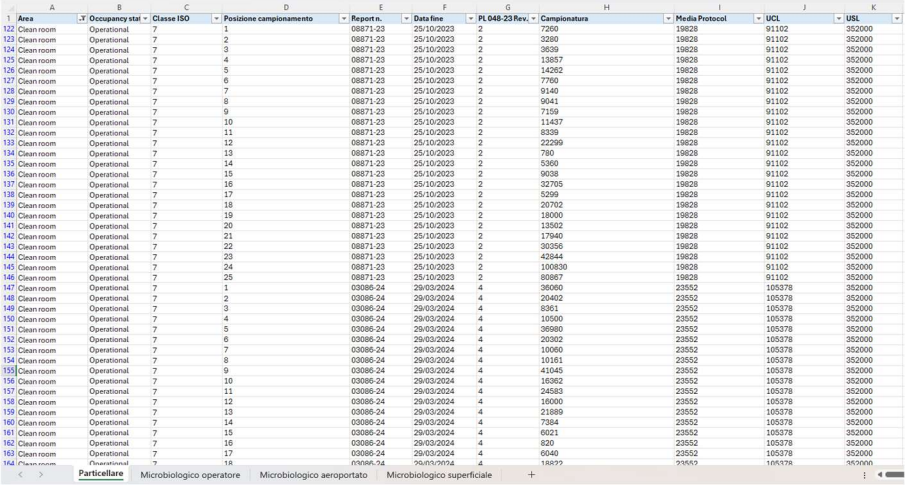


Figure 6.3 Example of the database structure used for the collection, organization, and management of environmental monitoring data.

In conclusion, the objectives initially defined for this thesis can be considered fully achieved. The work enabled the creation of a structured and continuously expandable database, the introduction of a statistical methodology for the objective evaluation of environmental monitoring data, and the identification of environmental trends, process variability, and capability levels across the monitored facilities. Beyond the specific results obtained, the project established a replicable methodological framework that can support future Quality Assurance activities, facilitate data-driven decision making, contribute to risk-based environmental monitoring strategies, and provide a foundation for the future integration of environmental and product quality information within SIDAM S.r.l.

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8 Appendices

8.1 APPENDIX A - Additional graphical analyses of the Bove facility

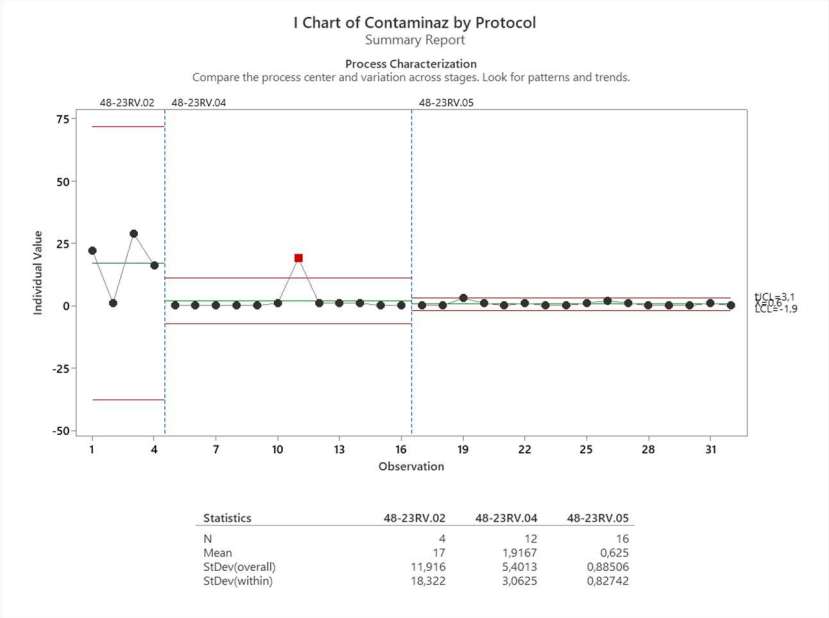


Figure 8.1 I-Chart of surface microbiological contamination measured in the Airlock environment of the Bove facility.

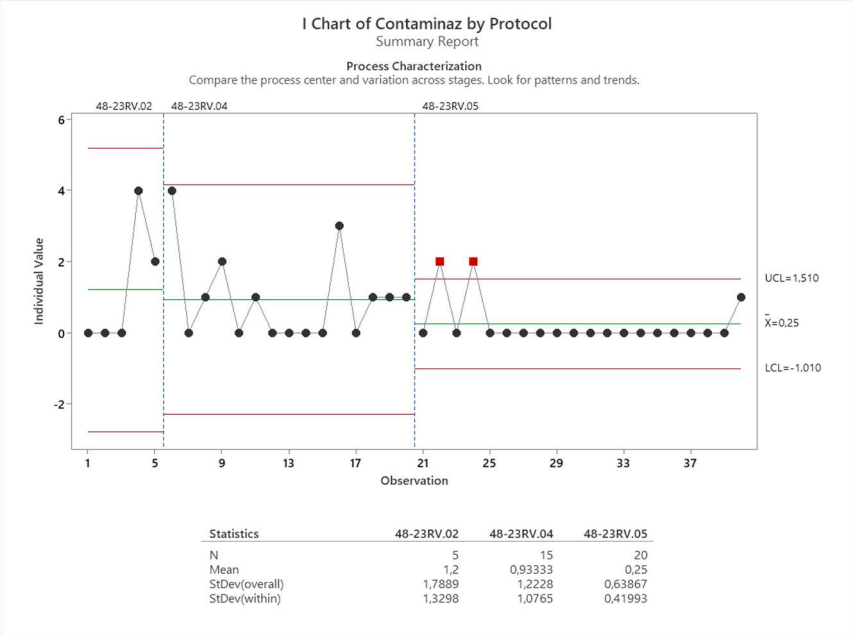


Figure 8.2 I-Chart of surface microbiological contamination measured in the Incoming Control Room environment of the Bove facility.

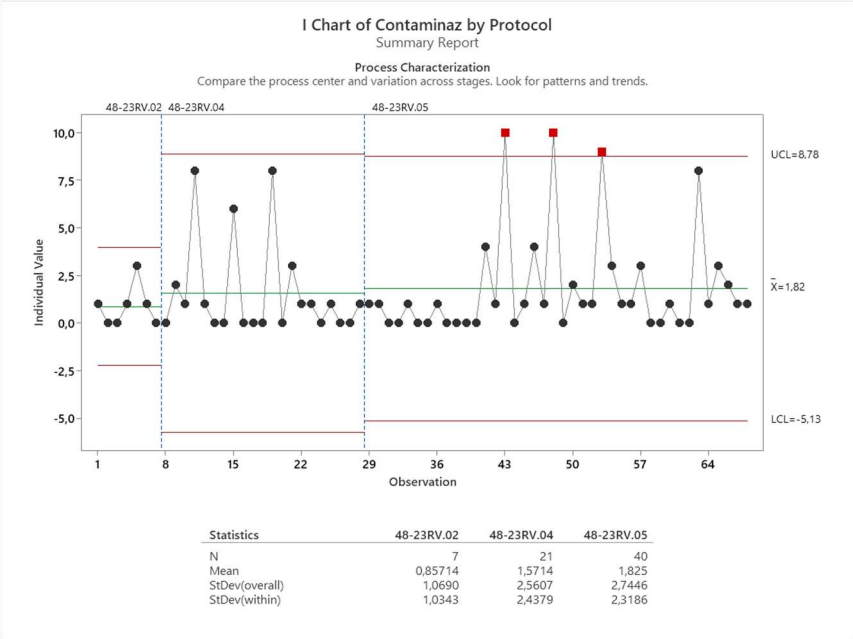


Figure 8.3 I-Chart of surface microbiological contamination measured in the Manufacturing Cassette Room environment of the Bove facility.

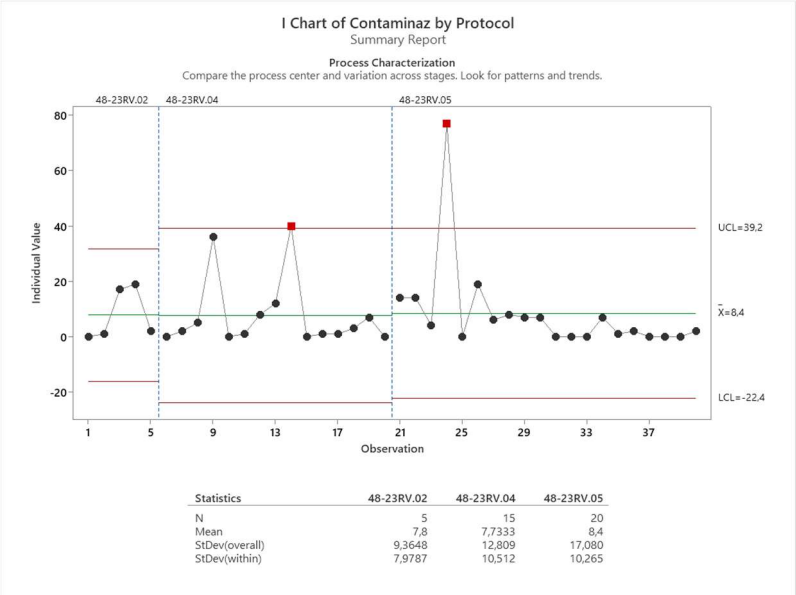


Figure 8.4 I-Chart of surface microbiological contamination measured in the Women's Changing Room environment of the Bove facility.

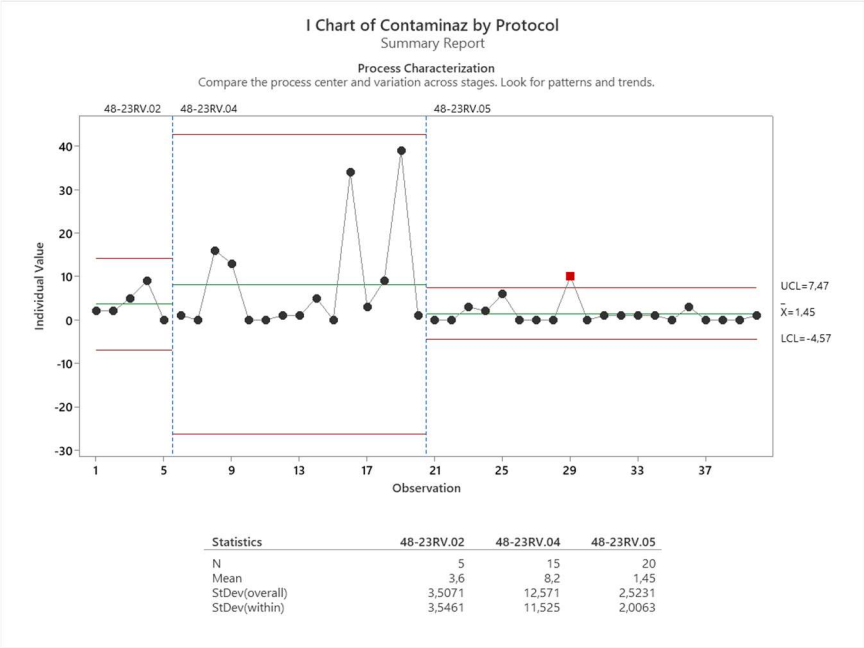


Figure 8.5 I-Chart of surface microbiological contamination measured in the Menn's Changing Room environment of the Bove facility.

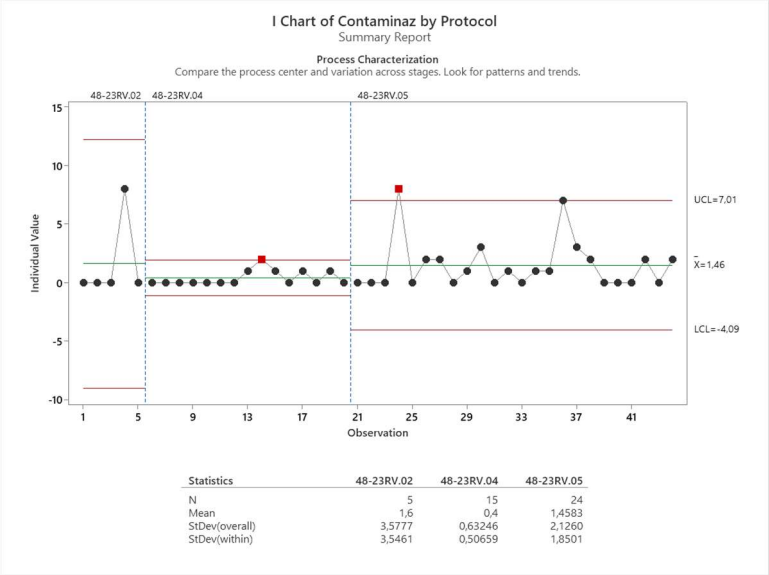


Figure 8.6 I-Chart of surface microbiological contamination measured in the Incoming Control Room environment of the Bove facility.

8.2 APPENDIX B - Additional graphical analyses of the Statale facility

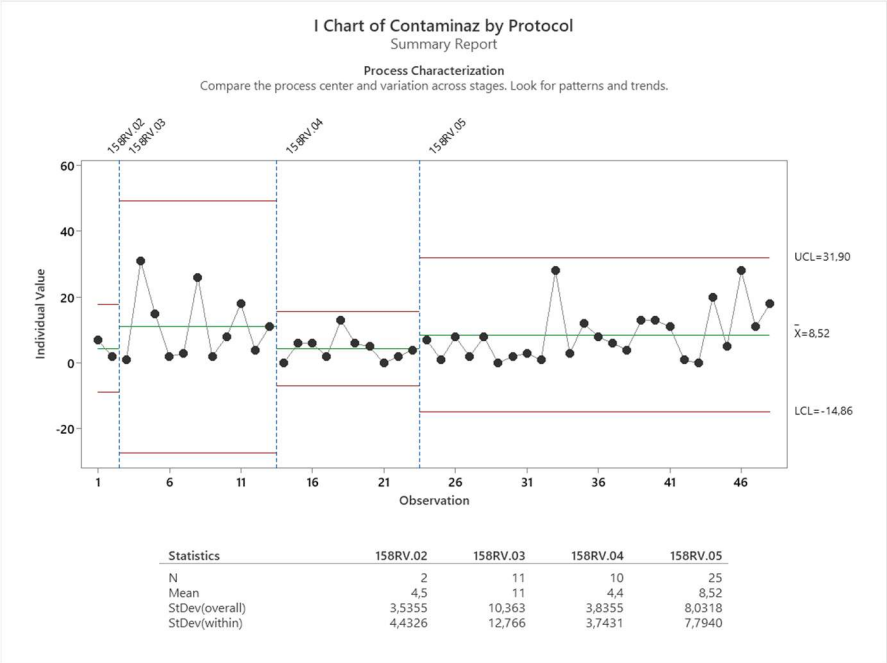


Figure 8.7 I-Chart of surface microbiological contamination measured in the Airlock environment of the Statale facility.

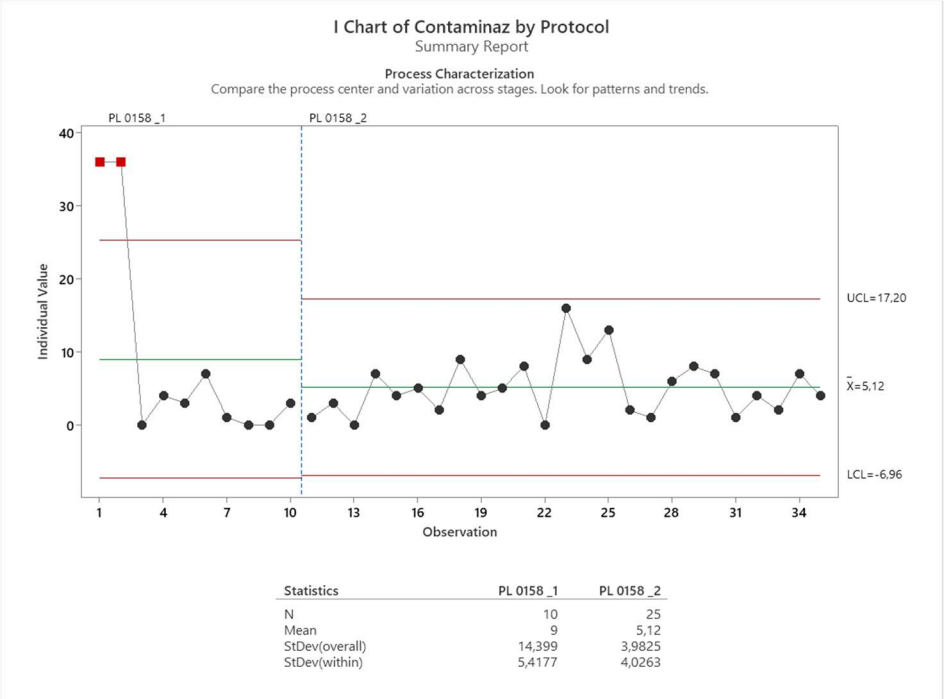


Figure 8.8 I-Chart of surface microbiological contamination measured in the Inlet Material Room environment of the Statale facility.

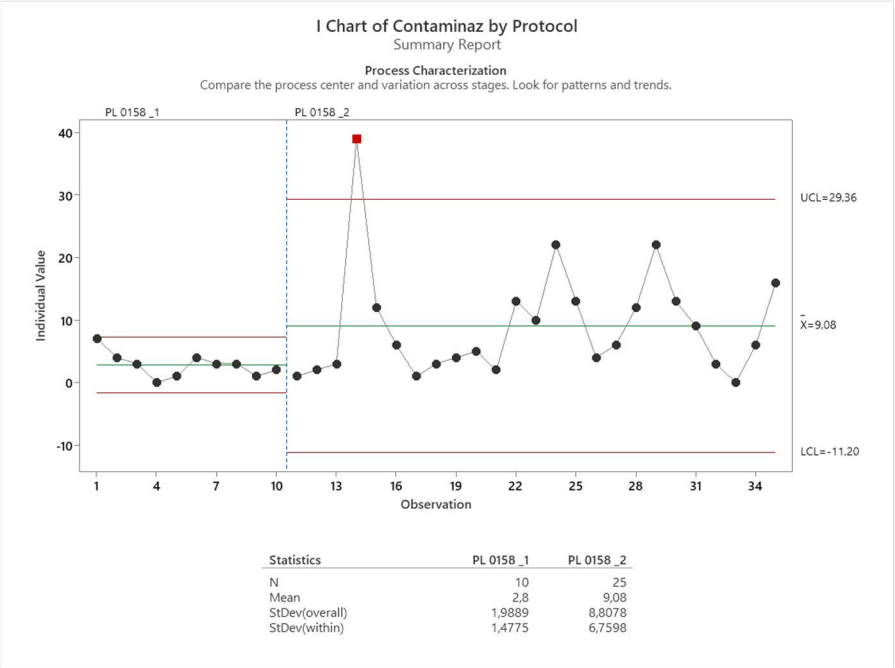


Figure 8.9 I-Chart of surface microbiological contamination measured in the Outlet Material Room environment of the Statale facility.

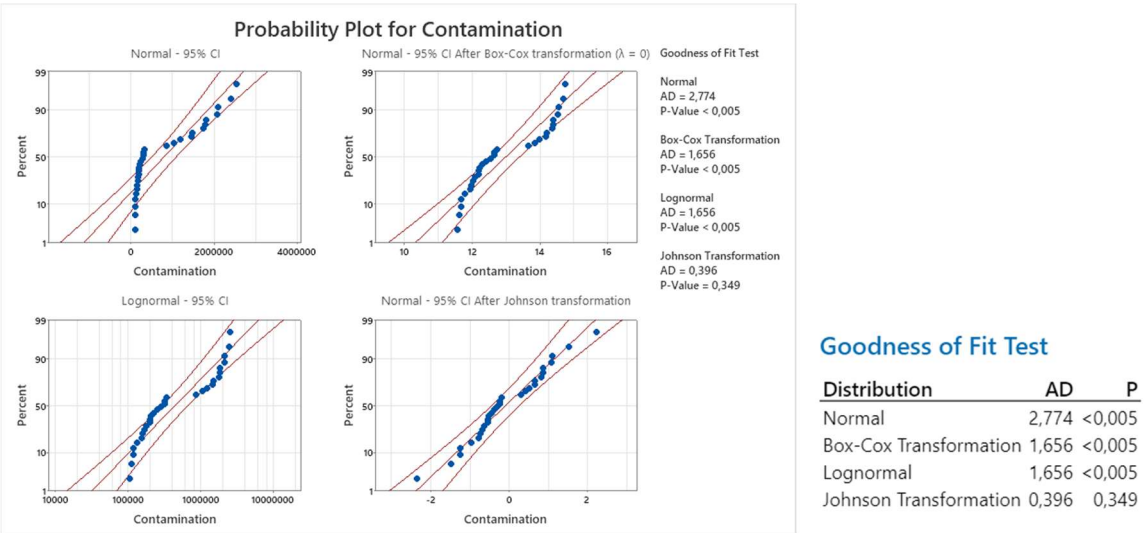
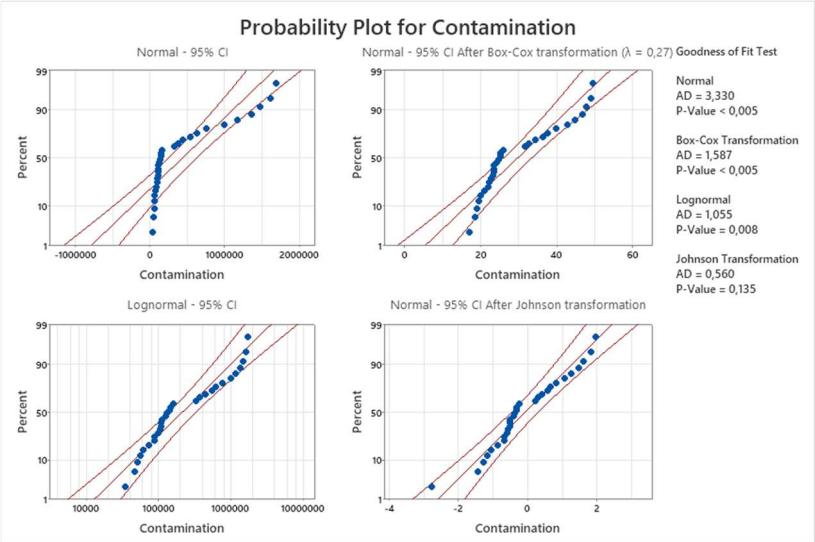


Figure 8.10 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the Airlock Dressing Room environment of the Via Statale facility for revision 05 of the PL0158 protocol.



Goodness of Fit Test

Distribution	AD	P
Normal	3,330	<0,005
Box-Cox Transformation	1,587	<0,005
Lognormal	1,055	0,008
Johnson Transformation	0,560	0,135

Figure 8.11 Probability plots and Goodness-of-fit test results associated with particulate contamination collected in the Airlock Washing Room environment of the Via Statale facility for revision 05 of the PL0158 protocol.

8.3 APPENDIX C – Additional statistical results

Table 8.1 Summary of the statistical analyses performed on the monitored environments not discussed in detail in the main Results chapter.

ID	ROOM	ISO	PL048-23 Rev.	One-Way ANOVA	2-Sample t Test	Cpk	Ppk	PPM>USL Exp. overall
19	Assembly Membrane room	7	02	0,003	0,007	Less data		
			04		0,121	/	3,66	0
			05					
18	Assembly Sensor Body room	7	02	0,093	/	/	/	/
			04		0,029	/	/	/
			05			/	7,53	0
16	Lot preparation room	7	02	0,001	0,001	/	/	/
			04		0,077	1,64	1,62	0,61
			05			3,25	2,28	0
24	Manufacturing Meeting room	7	02	0,01	0,196	Less data		
			04		0,001	2,91	2,25	0
			05			9,23	6,94	0
20	Siliconing Room	7	02	0,001	0,001	1,82	1,93	0
			04		0,001	/	6,81	0
			05			/	0,99	1528,60
22	Tub Cutting room	7	02	0,01	0,002	/	31,68	0
			04		0,022	/	1,25	0
			05			/	0,9	9,54
			02					

17	Warehouse For Lot Preparation	7	04	0,499	/	/	1,26	1,26
			05					
28A	Maintenance room	8	02	0,019	0,987	Less data		
			04		0,004	/	47,06	0
			05			5,17	3,12	0
	Pass environment	8	02	0,473	/	/	11,81	0
			04					
			05					
8	Assembly Cq Room (Via Statale)	8	02	0,001	0,001	/	1.18	203,89
			03		0,027	Less data		
			04		0,004	/	7,69	0
			05			/	2,35	0
6	Balance room	8	02	0,001	0,001	/	1.09	554,27
			03		0,002	Less data		
			04		0,006	Less data		
			05			/	1,80	0,04

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Desidero innanzitutto esprimere la mia più sincera gratitudine al mio relatore, il Professor Marco Mandolini, per avermi accompagnato e guidato durante l'intero percorso di tirocinio e di sviluppo di questa tesi. La sua costante disponibilità, la competenza e la capacità di trovare sempre il modo di offrire un supporto concreto sono state per me un punto di riferimento fondamentale, sia dal punto di vista scientifico che umano.

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