Research Article

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Microbial Monitoring of an Isolator in a Centralized Cytostatics Preparation Unit: The Experience of a Tunisian Hospital over a Period of Three Years

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Abstract

The Institute "Salah Azaiez" department of pharmacy ensures the preparation of injectable anti-neoplastics into a rigid isolator placed in a cleanroom ISO 8. The production area is submitted to routine microbial testing to verify its compliance with applicable requirements in terms of microbial contamination. Our objective is to evaluate the results of the microbial monitoring of the isolator over a period of approximately three years of functioning.

Materials and methods:

Control focuses on five emplacements into the isolator: working surfaces, isolator surfaces, gloves prints, air and critical areas. All results were collected and then analyzed.

Results and discussion: Positive cultures were found in 2.49% of cases.

Staphylococcus epidermidis and *Bacillus* sp. were the two predominant species.

Staphylococcus aureus and *Aspergillus* sp., two opportunistic pathogens, were also isolated. Number of non-conformities was particularly high during the first year of the study. This fact was correlated with a default of the sterilizing agent and a temporary malfunction of the isolator occurring during this period.

Conclusion:

Microbial monitoring of the production area is essential to ensure the quality of preparations. Results showed that the isolator wasn't always consistent with the GPP contamination limits. Meanwhile, non-conformities were in most cases correlated with failures occurring in the isolator.

Keywords: Isolator, Controlled Environment, Microbial Monitoring, Centralized preparation, Cytostatics

Introduction:

The Institute Salah Azaiez (ISA) is the reference center for surveillance, diagnosis and treatment of cancer in Tunisia. It was founded in 1969 within the framework of a franco-tunisian collaboration and is now recognized by the World Health Organization and the International Union against Cancer as a regional referral center for the diagnosis and treatment of breast and cervical cancer. The ISA has a capacity of 170 beds. To meet the need for quality, centralization and standardization, it was decided to prepare cytostatics in a dedicated unit adequately arranged for this purpose rather than in clinical departments. This unit, labeled "Centralized Cytostatic Preparation Unit" (CCPU), was created in 2008 and is part of the pharmacy department. The CCPU is under pharmaceutical supervision. A quality assurance system was put in place to ensure the quality of chemotherapy preparations.

The number of preparations until the end of the year 2012 was estimated at 70 preparations per month but it's prone to a great increase. Indeed, the process of centralization is currently undergoing extension in consultation with physicians in order to eradicate handling cytostatics by nurses in the clinical services. In order to comply with the requirements for parenteral preparations, an evaluation of the environment quality by measuring particular and microbial contamination levels is regularly achieved following a predefined schedule. The objective of the following article is to evaluate the experience of the CCPU with microbial contamination monitoring of the isolator over a period of approximately three years.

Materials and methods

CCPU description

Parenteral cytostatic drugs in the ISA are prepared in the CCPU. Isolator technology was chosen instead of vertical laminar air-flow hood. This type of completely enclosed workstation enables preparation in a sterile environment, avoiding aseptic faults¹. The CCPU is composed of a production room, a disinfection room and a storage area. The production room is an ISO 8 class environment equipped with a working rigid isolator (or main isolator), a transfer isolator and a rapid sterilizer. The working isolator is an ISO 4.8 closed and bacteriologically sealed volume, allowing isolation of the production area in relation to its environment. It offers four workstations dedicated to cytostatics handling, each one is equipped with double neoprene gloving portals. The working isolator is connected permanently and without breaking seal to the transfer isolator, which is used for the storage of sterilized drug products and medical devices. Moreover, the room production is equipped with a rapid sterilizer and two airlocks for releasing preparations and production wastes. The working isolator is in overpressure with respect to the production room, transfer isolator and airlocks. Access to the production room is allowed through a "personnel

airlock" and is restricted to qualified personnel adequately trained on proper aseptic gowning and garbing technique. The whole organization of the CCPU is intended to reduce the risk of microbial and particulate pyrogenic contamination.

Working methodology

Our study is retrospective including microbial results obtained over a period of approximately three years (from 15/02/2010 to 31/12/2012). Data were collected from the CCPU documentation and were used to perform a descriptive analysis.

Microbial control

The isolator has been in operation since January 2010 with periodically microbial control. Checks are carried out during its activity status in order to detect an unusual level of microbial contamination. In order to establish microbial monitoring parameters (sites, timing, frequency and sampling methods), we referred to two international guidelines because no national references were available:

1. The French versions of the Good Preparation Practices (GPP)² and the Good Hospital Pharmacy Practices (GHPP)³. These two guides specify that cytotoxic preparations must be performed in a controlled environment (laminar flow hood or isolator) and define monitoring methods and tolerated limits for biocontamination.
2. The French version of the International Standard ISO NF/EN 14698-1 and -2^{4,5}: dealing with the monitoring of microbial contamination in cleanrooms and associated controlled environments.

Also, the recommendations of the CCLIN⁶ (a coordination center for the fight against nosocomial infections in South-western of France) and the Aspec⁷ (the referral French organism dealing with the monitoring of cleanrooms and contamination) were taken into consideration.

Combining information from these different references, a plan was established and followed. Details of this plan will be explained later in the article. Besides, an approach based on a rationale and pragmatic analysis of the risk of contamination during the preparation process and a good understanding of the isolator conception was necessary for defining personalized monitoring terms (frequency, emplacements, critical sites and timing)⁸.

The sampling act is in charge of handlers and is carefully carried out in compliance with good practices². For this, handlers were prepared and trained for this task through specific courses under the supervision of the responsible pharmacist of the CCPU. This allowed us to avoid false positives and compare results over time.

Microbial control of the isolator focuses on air, isolator surfaces, working surface, glove prints and critical areas². Determination of air contamination was based on dividing the isolator into 9 areas, control is done by alternating even weeks (for even emplacements) and odd weeks (for odd emplacements)

Figure 1: Air sampling emplacements in the isolator. Note that emplacements 2, 4, 6 and 8 are controlled during even weeks and emplacements 1, 3, 5, 7 and 9 are controlled during odd weeks.

Figure 2: Critical emplacements chosen for microbial control of the isolator

[E1: bottom corner side handler 1; E2: low corner side handler 2; E3: bottom corner side handler 3; E4: bottom corner side handler 4; E5: high corner side handler 1; E6: high corner side handler 2; E7: top corner side handler 3; E8: high corner side handler 4; E9: top shelf; E10: bottom shelf; E11: seam of cuff side handler 1; E12: seam of cuff side handler 2; E13: seam of cuff side handler 3; E14: seam of cuff side handler 4; E15: seam of refrigerator 1; E16: seam of refrigerator 2; E17: seam of airlock 1; E18: seam of airlock 2; E19: door communicating with the transfer isolator T2; E20: door communicating with the rapid sterilizer].

These sites are particularly prone to a greater risk of microbial contamination. Two different swabs are realized per day of production. The twenty sites could be then entirely controlled after ten days. Methods used for detection of microbial contamination in the production area are simple and inexpensive. Four techniques are used for sampling^{2, 8, 9}.

1. Surfaces control using count-tact plates: it's a qualitative and quantitative method using Count-tact plates (Biomerieux®) which consist of a non-selective Trypticase Soya (TS) and Sabouraud (SC) mediums, respectively used to detect bacteria, mold and fungi. These plates, casting on a grid holder of 55 mm as diameter,

had a meniscus allowing direct application of the culture medium on a surface of about 25 cm².

2. Surfaces swabbing: it's a qualitative method in which microbial samples from the critical areas are obtained with a cotton swab immediately placed in a TS broth (Biomerieux®).

3. Gloves prints which is a qualitative and quantitative sampling method.

4. Air sedimentation known as a poorly sensitive qualitative and quantitative method. TS agar (Biomerieux®) of 90 mm as diameter is used for glove prints and air sedimentation methods.

All sampling plan details are shown

Sampling emplacement	Sampling method	Number of samples	Timing
Surface	Agar Count tact TS 55 mm Agar Count tact SC 55 mm	1 1	Before any disinfection and operation, prior to the beginning of production.
Air	Agar TS 90 mm	Pair control emplacements 2, 4, 6 and 8. Odd control emplacements 1, 3, 5, 7 and 9.	Place an appropriate number of petri dishes at the correspondent emplacements, when production begins and close them at the end of the process.
Working surface	Agar Count tact TS 55 mm	1 per working station	After handling and before cleaning.
Prints gloves	Agar TS 90 mm	1 per handler	At the end of production and before cleaning.
Critical areas	Swabbing	2	At the end of production and before cleaning.

Table 1: Sampling plan details (emplacements, timing, frequency and methods)[TS : Trypticase soja ; SC : Saboraud].

Samples are properly labeled (sample type, location, operator name and date sampling). Then, they are immediately sent to microbiology laboratory for analysis. After 14 days of incubation and daily checking, the CCPU receives the result report. A medium is considered positive if at least one colony is formed². In this case, the number and nature of microbial isolates are mentioned. Non-conformities are carefully registered in an Excel document created for the purpose. Registration includes the date, the emplacement, the sampler name, the number and nature of identified germs and the corrective undertaken action(s). The percentage of non-conformities per month is automatically calculated by the mean of Excel.

Results

A total of 5724 microbial samples is obtained over three years of isolator functioning. Positive cultures are found in 2.49% of cases. An important decline in the rate of positive samples was noted over time with a tendency towards nil during the end of 2012.

The isolator is a class A environment, a non-conformity is then defined as a number of Unity Forming Colony (UFC) ≥ 1 for all sample types (surfaces, air, critical areas and gloves prints)². Referring to this limit, 143 non-conformities are detected, distributed as follows: 26 for surfaces, 75 for air, 37 for print gloves and 4 for critical areas

Figure 3: Evolution of the percentage of positive samples in term of year and sample type.

Five species were isolated during the study period: staphylococcus epidermidis, staphylococcus aureus, citrobacter sp., bacillus sp.

and fungi aspergillus sp. Also, we note a drop in each of the five isolated microbial species between 2010 and 2012

Figure 4: Distribution of the number of non-conformities in term of year and isolated species.

This recession is more remarkable for staphylococcus epidermidis whose number decreased almost ten times but still however the predominant isolated seed followed by bacillus. Two reasons were chronologically identified and correlated with the unusual high number of non-conformities: a quality default of the peracetic acid (the sterilizing agent) and a minor damage in the specific neoprene gloves for chemotherapy. This defective sterilizing agent was responsible for the increase in the number of positive samples during the first year of study. The isolation of aspergillus sp. occurred mostly (4 out of a total of 5) during this same period.

Following the use of a new batch of peracetic acid provided by another supplier and controlled prior to utilization, the percentage of positive samples decreased obviously compared to 2010 but we still detected non-conformities from time to time. A miniscule perforation in one neoprene gloves occurring during the second year of study explained this non-compliance. As a remedy, we proceeded to the immediate change of the neoprene gloves at the concerned workstation.

Consequently, the microbial control of 2012 showed a significant decrease of contamination: only 0.5% of samples were positive versus 5.3% in 2010 and 1.3% in 2011. Only 4 non-conformities were found out during 2012 and no pathogens were identified in isolates. In addition, no contamination was detected in critical areas and surfaces.

Discussion

Chemotherapy preparations must comply with GPP guidelines² and satisfy to parenteral preparations requirements in terms of sterility and apyrogenicity (for preparations having a volume more than 15 ml to be administered at one time) as defined by United States Pharmacopoeia (USP)⁹ and European Pharmacopoeia (Eu.Ph)¹⁰ chapters. At the pharmacy department of the ISA, cytostatics are prepared under pharmaceutical supervision in an isolator which provides enclosed microenvironment¹¹. An isolator is a controlled environment area, which means a “defined area where sources of contamination are controlled using specified means”⁴. Isolator’s design prevents theoretically cross contamination between the handler and the product under preparation and between the product and the external environment^{12, 13}. Using such a close system with sterile and non-reusable medical devices allows the manufacture

of sterile therapeutic preparations with excellent confidence¹⁴. In these conditions, the risk of microbial contamination is considered low⁹. Once such a controlled environment is implemented, a cleaning and sanitizing program was established because simply using controlled environment does little to protect preparation and safety of patients if compliant cleaning and disinfection practices are not strictly adhered to⁹. In the other hand, measuring viable contaminant levels in the isolator provides a continuous feedback information on the performance and effectiveness of the sanitizing program⁹.

In the CCPU, cleaning and disinfection policies specify which cleaners and sanitizers to use, as well as areas to be cleaned and frequency of cleaning. Proper cleaning of the production room involves manual cleaning methods to reduce dirt and other foreign particulate matter on walls and floor, followed by chemical disinfection treatment to reduce contamination risk. Particular attention is paid to surfaces which are likely promoting microbial hand carriage (door handles and cupboards, etc...).

The walls and floor are cleaned with Surfanios® which is a microbicidal detergent and disinfectant recommended for the cleaning of floors, walls and equipment. As for scheduling, cleaning is frequently done. However, it’s best done during off-peak shifts, such as afternoons and weekends. In order to track long-term compliance, a form that documents the cleaning process was created. It includes the date and time, the area cleaned and disinfected, and the initials of the cleaning personnel. The working isolator is disinfected using a 0.25% dilution of Surfanios® before the beginning and at the end of a production day, using sterile non-woven compresses in order not to generate particles. It is critical to comply with the manufacturer-specified contact time, as this allows for proper microbicidal activity to take place. Thorough cleaning of the isolator followed by a wholly sterilization are realized every two weeks and eventually after an occurring malfunction. Cleaning of the isolator is performed by handlers who received appropriate training in proper cleanroom compliance practices. Handlers were also sensitized to the risk of contamination of parenteral medications and the impact on patient safety. Measures preceding access to the production room include correct hands washing followed by a sterile gloves wearing and correct dressing.

Given the prevalence of antibiotic-resistant microorganisms, such as Methicillin Resistant *Staphylococcus aureus* and Vancomycin Resistant *Enterococcus*, and the overall concern for nosocomial infections, it's recommended to make sanitizers and cleaning products rotate on a periodic basis¹⁵. Consecutively, cleaning programs are most effective and will help combat microorganism resistance in the cleanroom and the isolator. Unfortunately, this recommendation isn't yet in practice in the CCPU.

The results of the microbial control of the working isolator over a period of three years shows that identified germs don't deviate from those described in some other experiences of microbial monitoring. Thus, a similar study¹⁶ of microbial monitoring of a laminar air-flow hood class A located in a cleanroom class C showed an ecology where *staphylococcus* is predominant followed by *bacillus*. The detection of *aspergillus* sp. was also described and was correlated with a malfunction occurring in the isolator.

Another study of microbial validation of a class A hood in a hematology-oncology unit¹⁷ showed a substantially similar pathogen population in which *bacillus* sp., *penicillium* sp., coagulase-negative *staphylococcus* and fungi were predominant. *Staphylococcus epidermidis* and *bacillus* sp., the two most frequently isolated bacteria, are ubiquitous and saprophytic in nature. Their presence cannot therefore conclude on a specific source of contamination: air, manipulator or hardware can all be vectors of these germs.

However, the isolation of *citrobacter* sp., a seed usually related to the normal intestinal flora, is most likely related to a temporary failure in the cleaning and sanitizing measures. The presence of *staphylococcus aureus* and *aspergillus* sp., two opportunistic pathogens, is considered as alarming given the particular nosocomial and immunosuppression risk to which cancer patients are subjected. The way we proceeded given the high rate of non-conformities and the presence of pathogens was described by an internal procedure. Accordingly, some corrective measures involving all CCPU staff were chronologically undertaken as follows:

1. Revision of the cleaning and sanitization program including selection of a new microbicidal product, application method, and frequency. A strengthened cleaning and disinfection of the isolator was achieved using a more powerful disinfectant agent preceding the sterilization process. It was also decided to redefine the frequency of changing gloves during production. No improvement in microbial control results was shown consecutively, so we concluded that non-conformities weren't related to a failure in the sanitizing program.
2. Increased surveillance of personnel practices by supervisory staff and additional training on handling practices were indicated. A grid was used to assess compliance with good practices. Furthermore, a process simulation testing for aseptically filled products ("media fill test") was realized to verify the compliance of the production process and ensure that the handlers comply with GPP. Media fill test revealed negative so a non-aseptic preparation process was excluded. In the other hand, we controlled the sampling practices

by direct observation of handlers. No errors were noted so we became sure that positive cultures weren't due to an accidental contamination occurring during the sampling act.

3. The integrity of neoprene gloves of the four workstations was verified by visual inspection. No breakage of integrity was detected during the first year of isolator functioning (but during the second year).

4. The effectiveness of the sterilizing agent used (batch control) was verified. For this, commercially bioindicators were used according to the official compendial guidelines for sterility testing^{10, 11} (Eu.Ph and USP). A sterilization cycle was carried out in the presence of bioindicators. Then, bioindicators were cultured in a TS broth which revealed positive 48 hours later. We concluded that the sterilization process didn't destroy the microbial inoculum. Since parameters of the sterilization cycle (time and peracetic acid volume) were already settled during the initial validation of the sterilization process, it was concluded that the sterilizing agent itself was faulty. We proceeded then to order a new batch from the central pharmacy of Tunisia.

However, due to delays in the arrival, we didn't have the choice but utilizing the defective batch for a period up to six months (June 2010-December 2010), pending the arrival of the new product. It wasn't possible for us to use another available sterilizing agent (such as hydrogen peroxide) because the circuit of the isolator is exclusively adapted to peracetic acid. This period was correlated to the highest number of positive samples compared to the rest of the study period.

Conclusion

Microbial monitoring of the production area is essential for ensuring the quality of the preparations. Results over a period of approximately three years showed that isolator wasn't always consistent with contamination limits as set by the GPP. Meanwhile, some failures were identified and were correlated with the unusual levels of microbial contamination. Appropriate corrective measures were undertaken and helped restore microbiological compliance.

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