

Microbial contamination of syringes during preparation: The direct influence of environmental cleanliness and risk manipulations on end-product quality

CYRIL STUCKI, ANNA-MARIA SAUTTER, JOCELYNE FAVET, AND PASCAL BONNABRY

The preparation of sterile injectable preparations frequently necessitates a drug transfer or dilution step from a vial to the final syringe or bag. When the preparation is not performed in an aseptic environment, it is recommended that the drug be administered rapidly after reconstitution to avoid microbial contamination.¹ However, in some situations, drugs are prepared ahead of time and stored until they are needed. For instance, anesthesiologists often prepare syringes containing anesthesia-induction agents, neuromuscular blockers, and resuscitative drugs before they are used in surgical procedures.

The practice of storing drugs in hospital-filled syringes raises questions related to drug safety (i.e., might such storage adversely affect drug potency and allow for microbial growth of any contaminants introduced during preparation?). It is well-known that the contamination of syringes may increase the risk

Purpose. The direct influence of environmental cleanliness and risk manipulations on prepared syringes was evaluated.

Methods. Media-fill testing was used to estimate potential microbial contamination. Syringes were prepared in three different environments using four different uncontrolled high-risk manipulations. The three environments included an International Organization for Standardization (ISO) class 5 horizontal laminar-airflow hood in an ISO class 6 cleanroom (in accordance with *United States Pharmacopeia [USP]* chapter 797), an ISO class 7 drug preparation area of an operating room, and an uncontrolled decentralized pharmacy in a ward. For each combination of environment and manipulation, 100 syringes were filled by a single operator. The four high-risk manipulations used included simple filling of syringes with trypticase soy broth, three-second contact by the ungloved fingers of the operator with the hub of the syringe, three-second contact between an object and the hub of the syringe, and exposure of the filled syringes to ambient air for 10 minutes.

Results. Of the 1500 syringes prepared in three different environments, none pro-

duced within the cleanroom contained microorganisms, 6% were contaminated in the operating room, and 16% were contaminated in the ward ($p < 0.0001$). Certain high-risk manipulations were associated with a significant increase in the contamination of the surrogate syringes, including exposure to nonsterile ambient air and nonsterile objects or fingers ($p < 0.0001$).

Conclusion. High contamination rates were measured when the hub of syringes touched nonsterile environmental surfaces and fingers, whereas the drawn-air manipulation was associated with a lower risk of contamination. Working within a properly operating unidirectional airflow primary engineering control in an ISO class 5 cleanroom in accordance with *USP* chapter 797 requirements was demonstrated to be the best way to avoid bacterial or fungal contamination of injectable drugs directly resulting in patient infections.

Index terms: Aseptic areas; Compounding; Contamination; Injections; Laminar flow; Pharmacy, hospital; Standards; Syringes

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CYRIL STUCKI, PHARM.D., is Hospital Pharmacist; ANNA-MARIA SAUTTER, PH.D., is Hospital Pharmacist, Pharmacy, University Hospitals of Geneva, Geneva, Switzerland; JOCELYNE FAVET, PH.D., is Microbiologist and Teacher, Biology Department; AND PASCAL BONNABRY, PH.D., is Professor, Pharmacy Department, University of Geneva, Geneva, Switzerland.

Address correspondence to Dr. Stucki at the Pharmacy, Univer-

sity Hospitals of Geneva, Pharmacie des HUG, Rue Gabrielle-Perret-Gentil 4, CH-1211 Geneva 14, Switzerland (cyril.stucki@hcuge.ch).

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of infection, and several serious cases of such infection have been reported in the literature.²⁻¹³ To minimize the risk of end-product contamination, *United States Pharmacopeia (USP)* chapter 797 requirements limit the storage of drugs in prefilled syringes to a one-hour period.¹⁴

The two main factors that contribute to microbial contamination of drugs are the cleanliness of the work environment and the competency and care of the operator; however, no published data have quantified their respective impact. There is growing awareness that when proper procedures are followed, the provision of ready-to-use syringes can reduce the risk of dilution errors¹⁴⁻¹⁸ and that reduction of the contamination risks associated with the preparation of these drugs by competent personnel in cleanrooms, as an alternative to ward environments, is needed.¹⁹

During drug preparation, a variety of improper manipulations may compromise sterility, resulting in potential contamination of the end-product. Although both commonsense and operator training in aseptic technique for compounded sterile preparations (CSPs) clearly recommend against careless or non-standard manipulations, such practices do occur. In a previous study, we collected and tested unused syringes containing CSPs in our operating rooms and observed a 0.5% rate of contamination.²⁰ These data suggested that two syringes prepared in our hospital could be contaminated every day. This led our team to more thoroughly investigate the sources of potential contamination. The objective of this study was to estimate the probability of microbial contamination of syringes during preparation by simulating syringe-filling operations in three common hospital environments.

Methods

Media-fill testing was used to

estimate potential microbial contamination. A surrogate media-fill challenge incorporating a sterile trypticase soy broth^a (TSB) from a 100-mL vial was used to fill the test syringes.^b The TSB was received with the manufacturer's certificate of analysis, and the product's growth promotion and inhibition in closed syringes were validated in accordance with *USP* chapter 71 by inoculating the TSB with 10² colony-forming units (CFU)/mL of *Pseudomonas aeruginosa*,^c *Staphylococcus aureus*,^d *Bacillus subtilis* (spores),^e *Candida albicans*,^f and *Aspergillus niger*.^g Successful positive controls were those that demonstrated positive growth of the test inoculum in the TSB within three days (for bacteria) or five days (for fungi). To establish positive growth, each test syringe was compared to control samples. Transfer from a vial to syringes was simulated by substituting TSB for a drug. In this exercise, 5 mL of TSB was withdrawn from a 100-mL vial and injected into a 10-mL syringe. Five milliliters of filtered air was aseptically added to each syringe through a sterile 0.22- μ m air filter in order to support the potential growth of aerobic contaminants. The syringes were then sealed with a Luer-Lok cap. In accordance with *USP* chapter 797, these syringes were incubated for seven days at a mean \pm S.D. temperature of 25 \pm 1 °C and for seven more days at 32 \pm 0.2 °C.

After 14 days, all samples were analyzed by direct examination with incandescent electric light, and positive contamination was declared when any turbidity of the growth medium was observed. Syringes were prepared in three different environments employing four different uncontrolled high-risk manipulations. For each combination of environment and manipulation, 100 syringes were filled by a single operator. This operator received training on aseptic preparation techniques validated through individual media-fill tests.

The three testing environments were (1) an International Organization for Standardization (ISO) class 5 horizontal laminar-airflow hood in an ISO class 6 cleanroom, (2) an ISO class 7 drug preparation area of an operating room, and (3) an uncontrolled decentralized pharmacy in a ward. Air particulate contamination of the three sites was determined with a discrete particle counter (DPC).^h This DPC is annually calibrated by the manufacturer in accordance with international standards. The DPC was employed to estimate the total airborne particulate contamination burden as ≥ 0.5 - and ≥ 5.0 - μ m particles per cubic meter. Concomitant aerobiological testing was not conducted.

Four common high-risk manipulations were investigated in this study, and the results were correlated with a simple filling of syringes with TSB. This exercise was considered the baseline contamination risk. This operation consisted of aseptically withdrawing 5 mL of TSB from a 100-mL vial with a sterile 10-mL syringe and a sterile spike. One-hundred syringes were filled in this manner for each of the environments and conditions tested. A total of 1500 surrogate syringes were produced by a single operator to limit variability due to technique. During the first high-risk manipulation, the TSB was aspirated into the syringe and left uncovered, exposing the content to air. This manipulation is often observed in a ward or operating room to adjust the volume of a drug in or to remove air bubbles from a syringe. After filling the syringe with TSB, 5 mL of air was drawn into the syringe three times using the plunger. The second exercise evaluated the risk of contamination after three seconds of contact by the operator's ungloved fingers with the hub of the syringe. This manipulation clearly compromises sterility; however, this kind of manipulation can accidentally occur. The third exercise simulated contact

between an object and the hub of the syringe. During this exercise, each contact, lasting three seconds, was made with the following objects normally located within the representative environments: the walls and floor of the laminar-airflow hood in the cleanroom, trays usually used by anesthetists to store the syringes in the operating room, and nearby objects such as a table or vials in the ward. Contamination rates of the surfaces used for the contact were estimated with a microbial Rodac surface-contact plate count using a casein-peptone soymeal-peptone agar plate.¹ While this manipulation is strictly forbidden during drug preparation, it was used to demonstrate the result of contact between syringes and surfaces, which could occur in certain situations (e.g., when the protective cap or needle is accidentally disconnected). The fourth high-risk manipulation investigated was the undisturbed exposure of the filled syringes to ambient air for 10 minutes. This potential breach of sterility occurs when the cap or needle is separated from the syringe body (i.e., during a disturbance or shifting of syringes). Fisher's exact test was performed to determine if significant differences existed among the different environments and high-risk manipulations.

Results

The viability of microbes present

in the test syringes was confirmed by the inoculation test. In all cases, growth was visible after three days. Of the 1500 syringes prepared in three different environments, none prepared in the cleanroom contained microorganisms, 6% were contaminated in the operating room, and 16% were contaminated in the ward ($p < 0.0001$) (Table 1). These results correlated with the amount of airborne particulate matter measured (Table 2). The contamination in the laminar-airflow hood was null, whereas the drug preparation area of the operating room had particulate level requirements for an ISO class 7 cleanroom, and the ward was an ISO class 8 cleanroom. Certain high-risk manipulations were associated with a significant increase in the contamination of the surrogate syringes ($p < 0.0001$). No contamination was observed when the syringes were simply filled controls or when air alone was drawn into the syringe. When the syringes were exposed to nonsterile ambient air for 10 minutes, a contamination rate of 1% was observed. Comparatively high rates of contamination were observed when contact occurred with nonsterile objects or fingers. Contact of the syringe lumen with an object in the operating room and the ward resulted in contamination rates of 3% and 67%, respectively, correlating with the number of CFUs revealed by plate counts for each environment (Table

2). In situations where the syringe lumen contacted the ungloved fingers of the operator, contamination rates of 24% and 10% were observed in the operating room and the ward, respectively.

Discussion

Although the resistance of drug solutions to the growth of bacteria and fungi has been previously investigated, few studies have assessed the risk of microbial contamination during the standard preparation of syringes in a clinical environment.^{21,22} The current study was performed to estimate the risk of contamination when syringes were prepared with high-risk manipulations and stored in an operating room or a ward. Microbial contamination of syringes containing sterile media correlates with both the rate of environmental contamination (i.e., air and surfaces) and the occurrence of high-risk manipulations. An ISO class 5 cleanroom is a highly aseptic environment, and our study confirms the efficacy of this level of control when proper equipment and well-developed procedures are in place. None of the syringes prepared in the cleanroom were contaminated by microorganisms, even after sustaining contact with previously disinfected surfaces of the horizontal laminar-airflow hood or sterile gloves.

This study illustrates the importance of proper handling of drugs

Table 1. Rates of Syringe Contamination by Environments and Types of Manipulation (n = 1500)

Environment ^a	% Contamination by Type of Manipulation					Total % Contaminated Syringes
	Simple Filling	Air Introduced Into Syringe	Syringe Without Cap	Syringe Tip in Contact With Fingers	Syringe Tip in Contact With Object	
Cleanroom ^b	0	0	0	0	0	0
Operating room	0	0	1	24	3	6
Ward	0	0	1	10	67	16
Total %	0	0	1	11	23	7

^an = 100 for each condition and type of manipulation; the total number of syringes tested was 1500.

^bHorizontal laminar-airflow hood in International Organization for Standardization class 5 cleanroom.

Table 2.

Microbial and Particulate Contamination Rates by Environment

Environment	Mean \pm S.D. No. Particles/m ³ (n = 8 per site)		Mean \pm S.D. No. Colony-Forming Units/Agar Plate Count (25 cm ²) (n = 20 plates per site)
	$\geq 0.5 \mu\text{m}$	$\geq 5 \mu\text{m}$	
Cleanroom ^a	0 \pm 0	0 \pm 0	0 \pm 0
Operating room	56,626 \pm 17, 173	2,908 \pm 673	2 \pm 5
Ward	1,644,537 \pm 162, 466	16,430 \pm 19, 541	10 \pm 5

^aHorizontal laminar-airflow hood in International Organization for Standardization class 5 cleanroom.

in an appropriate environment when an extended period of storage is expected (i.e., in an operating room) or when a highly sensitive administration route is involved (i.e., intrathecal). Results from the other environments tested confirm a higher risk but also demonstrate a much larger rate of contamination when the syringe hub touched a nonsterile surface than when contact with ambient air occurred. No bacterial growth was observed in syringes that were simply filled, which was expected, as a single fluid transfer is not considered a high-risk manipulation.

Although uncontrolled or poorly controlled air is commonly thought to be a source of contamination, our results indicate that direct aerobiological contamination is rare. The small volume (5 mL) of air introduced through the hub of the syringe, as well as the relative cleanliness of the testing environments, may explain these results. However, when the unprotected syringe comes into contact with nonsterile ambient air for an extended period of time, contamination is possible, though not frequent. Therefore, as a best practice, it is recommended to systematically cover syringes and needles with a sterile cap as soon as dose preparation is completed. Contact between the hub of the syringe and an object resulted in a high rate of contamination in the operating room and in the least clean environment, the ward. Similarly, we found a high rate of microbial contamination

when the ungloved fingertip came into contact with the hub of the syringe. Both of these types of breaks in aseptic technique are considered high risk, and health care workers must be made aware of this fact during their training and education.

Simple measures can be applied to reduce these risks, such as those defined in *USP* chapter 797.¹⁴ The best practice is to discard syringes whenever contact with any surface accidentally occurs. It should be possible to establish a formal link among airborne particle counts, the number of CFUs per plate, and syringe contamination results, but in our study, the number of environmental controls was too few. However, there appears to be a clear correlation among these variables in the cleanroom, the operating room, and the ward. The growth medium we used supports the development of a large number of microbial species; however, it does not promote growth of all organisms, a factor that may have slightly diminished the actual results.

Our study did not reveal any surprising results but illustrated the extent of the risk associated with the practices surveyed, allowing us to better understand the level of risk that these manipulations pose and the direct effect of sterility in the compounding environment on end-product quality. These data are useful to sensitize health care workers to this pivotal issue during their training and to demonstrate the advantages of compounding sterile preparations in

cleanrooms in accordance with *USP* chapter 797 and other standards.

Conclusion

High contamination rates were measured when the hub of syringes touched nonsterile environmental surfaces and fingers, whereas the drawn-air manipulation was associated with a lower risk of contamination. Working within a properly operating unidirectional airflow primary engineering control in an ISO class 5 cleanroom in accordance with *USP* chapter 797 requirements was demonstrated to be the best way to avoid bacterial or fungal contamination of injectable drugs directly resulting in patient infections.

^aTSB-TS, BioMérieux SA, chemin de l'Orme Marcy l'Étoile France, lot 44011.

^bKlerpack Shield, Medicure, Aldershot, United Kingdom, lot 4109SW5D.

^cAmerican Type Culture Collection, Manassas, VA, ATCC 9027.

^dAmerican Type Culture Collection, ATCC 6538P.

^eAmerican Type Culture Collection, ATCC 6633.

^fAmerican Type Culture Collection, ATCC 10231.

^gAmerican Type Culture Collection, ATCC 16404.

^hMet One Skan, Basel, Switzerland.

ⁱHeipha Diagnostika, Eppenheim, Germany.

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