Robotic reconstitution of cytostatic drugs and monoclonal antibodies: transforming aseptic drug compounding in hospital pharmacies

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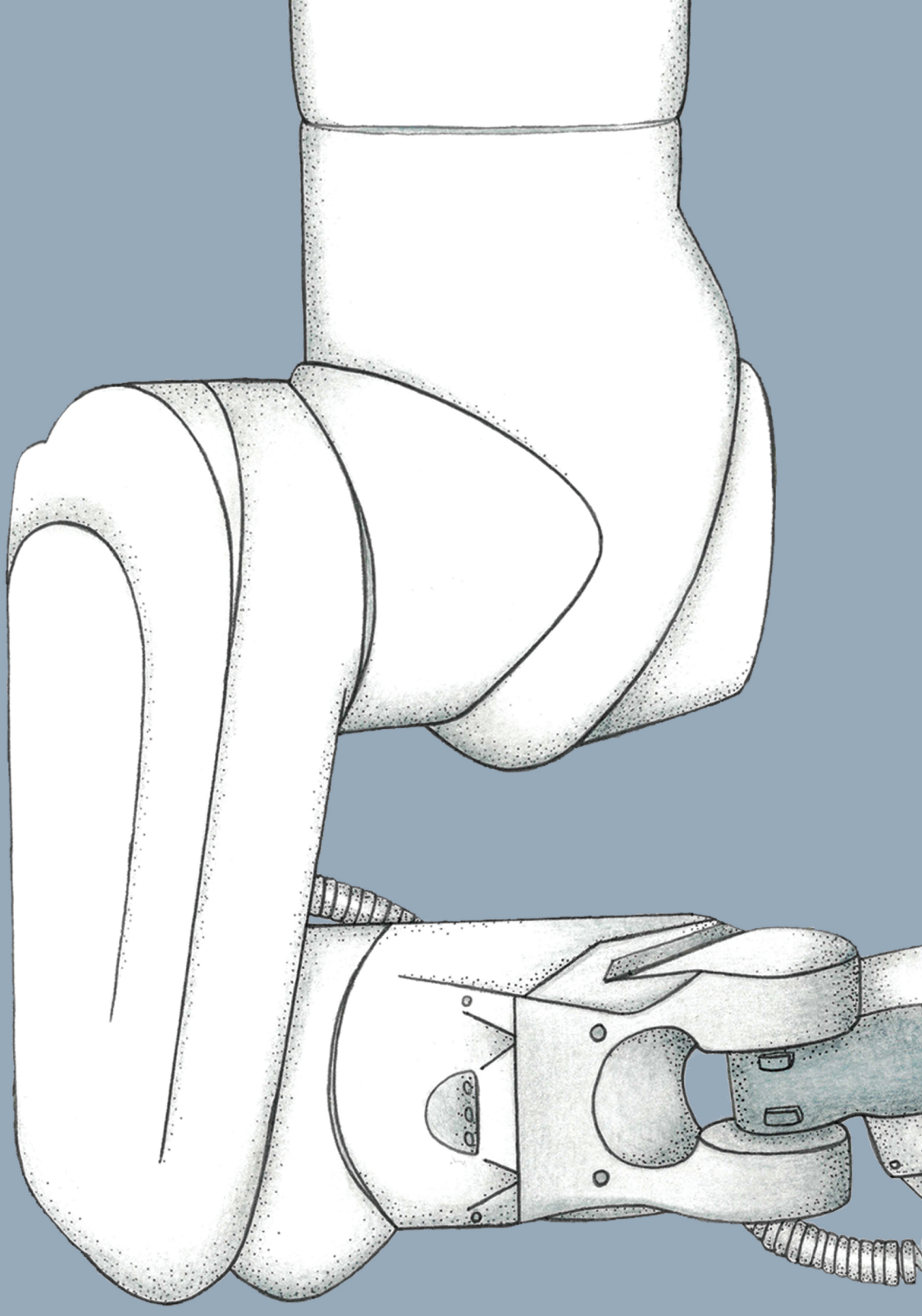

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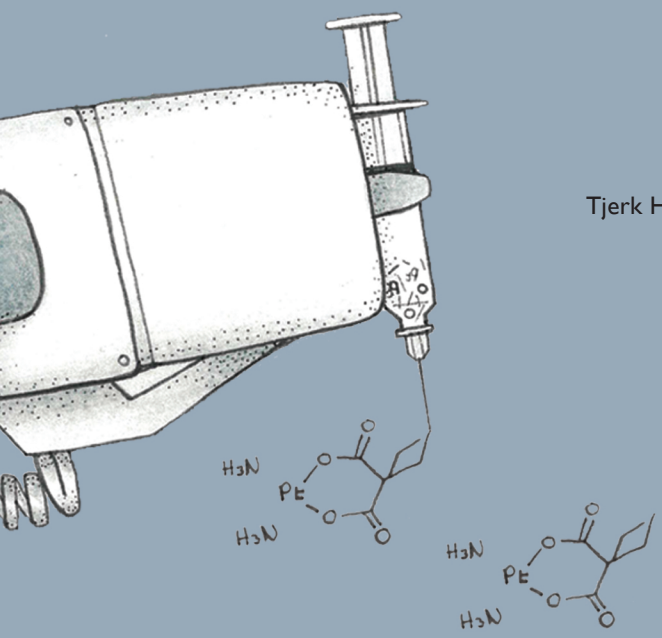
Microbiological performance of a robotic system for aseptic compounding of cytostatic drugs



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ABSTRACT

Background Compounding of cytostatic drugs requires strict aseptic procedures, while exposure to toxic drugs and repetitive manual movements should be minimized. Furthermore, reuse of vials is desirable to lower the costs. To assess if all this might be safely achieved with a robot, this study aimed at qualifying the aseptic preparation process with the robotic system APOTECAchemo.

Methods The aseptic compounding of patient-individual cytostatic solutions was simulated with media fill simulation tests to qualify the performance according to European GMP Annex 1. The contamination in the environment was measured in critical places using settle plates, contact plates, active air sampling and particle counting. Media-fill simulation tests were prepared in 3 production batches. The second part of the study evaluated the microbiological shelf-life of commercial drug vials after repeated puncturing. On six days, fifty syringes of 15 ml media were prepared from the same 50 vials with the robot. After each preparation, vials were covered with an IVA seal upon unloading from the robot to protect them from microbiological contamination.

Results No microbiological contamination was found in any of the 96 media fill preparations, nor in any of the 300 syringes that were prepared with repeated puncturing. The compounding area met class A limits, while class A criteria were not fulfilled by the contact plates and settle plates placed on the right side of the loading area. There, the average colony forming units (cfu) were 3 and 1.17, respectively, meeting class B criteria.

Conclusions Robotical compounding of cytostatic drugs with APOTECAchemo meets the microbiological requirements of the European GMP. In addition, the robot can reuse vials repeatedly and safely, thereby enabling extended usage.

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INTRODUCTION

Compounding of cytostatic drugs requires strict aseptic procedures, while exposure to toxic drugs and repetitive manual movements should be minimized. In the beginning of this millennium, the first fully automated robots for preparation of chemotherapy admixtures were introduced in hospital pharmacies.[1,2] Although several options for non-toxic drug compounding have become available since, the main focus has remained on cytostatic compounding robots. Underlying reason is that automating the preparation of intravenous drugs would appear most beneficial for those drugs where human errors pose the greatest risk for patients. In addition, shielding hospital pharmacy staff from exposure to hazardous drugs has also driven this innovation forward.

Microbiological stability of parenteral drugs remains a source of concern for pharmacists, as was demonstrated in a recent meta-analysis on aseptic technique studies in clinical and pharmaceutical environments.[3] This meta-analysis demonstrated a benefit for compounding in hospital pharmacies over clinical environments, but contamination rates in the pharmaceutical environments remained > 0%. Hence, reducing the microbiological burden of sterile injectable preparations as much as possible is pivotal, especially for oncology patients, who have a high risk of severe health injury from infection. Also to this end, automation of the aseptic process, where human contact with the products is minimized, can be beneficial.[4] In addition to microbiological cleanliness, dosing accuracy of the compounded cytotoxic drugs is of the upmost importance as well. This aspect of robotic compounding has been described and validated extensively by us and others, and is described elsewhere.[5-7]

In 2016 the OLVG hospital purchased a cytotoxic compounding robot (APOTECAchemo, Loccioni, Italy). The major aims were to minimize both the repetitive movements in aseptic procedures for the pharmacy technicians and the number of full-time equivalents needed for the routine cytostatic process, since there is a shortage of qualified technicians in the Amsterdam region. Furthermore, aims were a reduction of possible exposure of staff members to traces of cytotoxic drugs, as well as a reduction of the possibility of drug errors. Finally, the option to reuse vials of expensive drugs was examined, which is prohibited in the manual process by the Dutch national inspection authorities, but could greatly reduce the increasing costs of anticancer therapy.

Before using the robot in daily pharmacy practice, a thorough validation procedure under GMP was carried out. An important part of the validation procedure was the evaluation of the microbiological performance of the robotic system. As yet, limited data have been published on the microbiological safety of compounding robots. Of

the first generation robots, no data are available on media fill simulations that have been submitted to peer reviewing. Of the second generation of robots, one study was published in abstract form, measuring contamination in media fill simulations in combination with particle measurements. This study showed no growth in 108 consecutive simulations.[8] A second study on media fill simulations concluded that the robot complied with the PIC/S requirements for validation of the aseptic process[9] as well as with the pharmacopeia monograph on pharmaceutical compounding.[10,11] To this date, no study investigated repeated puncturing in combination with media fill simulations in a cytotoxic compounding robot.

Our study aimed to determine whether APOTECAchemo can perform individual compounding of parenteral solutions without risk of microbiological contamination. In addition, our aim was to determine whether the robot can reuse vials repeatedly and safely.

METHODS

Setting

This study was conducted in the pharmacy-based, centralized cytotoxic drugs preparation unit of the OLVG hospital in Amsterdam, The Netherlands. The OLVG comprises 48 inpatient beds and 17 outpatient seats. Cytostatic products such as infusion bags, elastomeric pumps, and ready-to-administer syringes are prepared in a biological safety cabinet (BSC) class A and in the robotic system APOTECAchemo (Loccioni, Italy), placed in the same Grade C cleanroom with negative air pressure (−5 Pa). The annual workload amounts to 13.000 cytostatic preparations.

The robotic system is designed for patient individual ready-to-use parenteral doses and consists of a loading area and a compounding area. The pharmacy technician loads the starting materials (drug vials, intravenous fluid bags, syringes, elastomeric pumps and needles) and unloads the finished products, which are both temporarily stored in a rotating warehouse. All drug vials are identified by photo recognition and height, and are weighed inside the compounding area of the robot. The robotic arm transfers the necessary components to the compounding area characterised by a negative air pressure gradient and vertical laminar airflow. This area refers to a Grade A cleanroom environment.[12] In the compounding area, the robotic arm prepares the individual doses using gravimetric quality control.

At the end of each workday, the total inside of the robot (compounding and loading area) is cleaned manually with isopropyl alcohol 80% (Clinisteril, Fresenius Kabi, Zeist, the Netherlands). An extensive cleaning procedure, also covering the total inside of the robot is performed weekly with sterile soapy water (Klercide neutral detergent, Ecolab, Minnesota, USA) followed by biocide C (6% hydrogen peroxide, Ecolab, Minnesota, USA).[13] After cleaning, UV irradiation is used daily for 4 h as was shown to be effective previously.[14]

We performed a validation of the robot aseptic process by performing media-fill simulation preparations on three independent maximum batch sizes in our setting, as is the requirement for small batch size compounding (< 3000 units) in the GMP annex 1.[12] The batches consisted of combinations of all possible critical steps in the compounding process. During the validation process, passive air monitoring with settle plates, surface monitoring with contact plates, surface air system tests and continuous particle measurements were performed on predefined locations inside the compounding area of the robot. In addition, we tested the microbiological safety, in a worst-case scenario, of repeated puncturing of drug vials.

Performance qualification of the aseptic process

On three separate days, 32 infusion bags ranging from 50 to 250 ml (NaCl 0.9% Baxter Viaflo) were automatically prepared by three different pharmacy technicians using the robot. The pharmacy technicians were instructed to imitate the routine production as closely as possible. To simulate the full range of possible preparations in real clinical practice, different combinations of critical compounding steps were performed: dissolving of drug vials with powder, withdrawing volume in a syringe, withdrawing an amount of vehicle solution from an infusion bag, adding concentrated media to an infusion bag, and filling an empty bag with non-concentrated media. The aseptic process was simulated by using tryptic soy broth (TSB) as growth media in lieu of cytotoxic drugs: 4× concentrated 10 ml and non-concentrated 100 ml (Biotrading, Utrecht, the Netherlands). The firm Biotrading is subject to annual auditing by the Dutch Pharmacy Association and each batch of TSB is tested to comply with the growth requirements of the European Pharmacopeia.[15] The 4× concentrated TSB was reconstituted with water for injection (Fresenius Kabi, Zeist, the Netherlands). All vials and stoppers were disinfected with 80% isopropylic alcohol (Clinisteril, Fresenius Kabi, Zeist, the Netherlands) by the operators in the loading area of the robot. Negative controls (unopened tryptic soy broth) and positive controls (contaminated by coughing on the soy broth) were included in the analysis. The acceptance criterium for the media fill simulation tests was zero growth.

Repeated vial puncturing

To test the microbiological performance of repeated vial puncturing, 50 non-concentrated 100 ml TSB vials (Biotrading, Utrecht, the Netherlands) were used to prepare 50 syringes of 15 ml (Drug Compounding Dosing Device ASN-20 Loccioni, Ancona, Italy) on day 1. Before compounding, rubber stoppers were disinfected with 80% isopropylic alcohol (Clinisteril, Fresenius Kabi, Zeist, the Netherlands) in the loading area of the robot. After compounding, the rubber stoppers were covered with an IVA seal (Covidien, Dublin, Ireland) in the unloading area of the robot and stored in the refrigerator outside of the cleanroom. The 50 vials punctured on day 1 were used again to prepare 50 syringes on each of the following days: 2, 3, 6, 7 and 8. Hence, each vial was punctured 6 times. After each batch preparation, a new IVA seal was used to cover each individual vial. In total, 300 syringes were prepared in this way, enabling a certainty of<1% contamination with a 95% confidence interval (0 positive in 300 units). Again, negative controls (unopened tryptic soy broth) and positive controls (contaminated by coughing on the soy broth) were included in the analysis. The acceptance criterium was zero growth in any of the media filled syringes.

Particle counts

A validated particle counter (Lighthouse Cleanroom Technology, Boven-Leeuwen, the Netherlands) was placed close to the dosing device where the compounding is performed, as this is the most critical spot in the compounding area. Continuous particle counting was performed during the entire media fill simulation test runs. The acceptance criteria for Grade A were: <3250/m3 for particles up to 0.5 µm and <20/m3 for particles up to 5 µm, both at rest as well as in operation.[12]

Air and surface microbiological monitoring (Fig. 1)

During the performance qualification, settle plates (TSA, Tryptone Soya Agar, Biotrading, Utrecht, the Netherlands) were placed and opened at six critical areas in the robot. In addition, the air was monitored twice daily, at the end of both shifts, using surface air system (SAS) tests. Herewith one cubic meter of air was sampled from four critical zones in the robot, and conveyed to contact plates loaded with TSA. Finally, surface monitoring was performed by using contact plates (Rodac, Biotrading, Utrecht, the Netherlands) on 13 predetermined critical areas. The acceptance criteria for air and surface monitoring for grade A were:<1 cfu (colony forming unit) on average for the 1 cubic meter SAS samples, and <1 cfu on average per location for the contact plates and for the settle plates during 4 h of opening.[12]

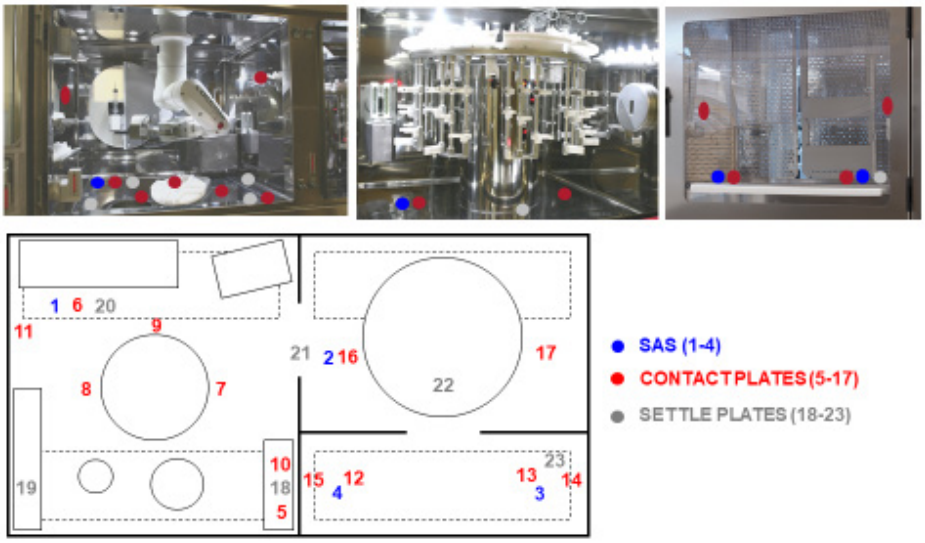

Fig. 1. Microbiological monitoring locations in the robot.

Incubation and analysis

All media fill products, as well as all settle and contact plates, were incubated for 14 days at 30 °C, to enable growth of both bacteria and fungi. Media fill products were visually inspected for turbidity at day 7 and day 14 by qualified staff from our quality control laboratory. All plates were inspected at day 7 and day 14, and cfu were counted when present. Air particle measurements were stratified according to particle size (up to 0.5 µm/m3 and up to 5 µm/m3).[12] Data were analysed using Excel (Microsoft Office, 2016, Microsoft, Redmond, USA).

RESULTS

Media fill simulation tests and repeated puncturing

No contamination was found in any of the 96 media fill preparations, nor in any of the 300 syringes that were prepared with media in the repeated puncturing study.

Particle counts

Particle counts were performed continuously on the three days of the media fill simulation tests. The robot was utilized from 9:00 to 17:00, with a break between 12:00 and 13:30. During the breaks, particle counts revealed no particles as expected (at rest). During operation, particle counts were well below the criteria for grade A. Detailed results are presented in Table 1.

Table 1. Results of the particle counts in the compounding area "in operation".

	0.5 µm particles			5 µm particles		
	Min-max (counts/m^3)	Mean (counts/m^3) ± st dev	Norm (counts/m^3)	Min-max (counts/m^3)	Mean (counts/m^3) ± st dev	Norm (counts/m^3)
Day 1	0-190	12.6 ± 42.6	<3250	0-2	0.01± 0.13	<20
Day 2	0-652	4.1 ± 34.0	<3250	0-4	0.09 ± 0.60	<20
Day 3	0-201	3.0 ± 20.8	<3250	0-6	0.02 ± 0.32	<20

Air and surface microbiological monitoring

On three different days during the media fill simulation tests, four areas inside the compounding area of the robot were sampled twice (at the beginning and at the end of each compounding run) with the SAS. At the beginning of each run, 0 cfu was found on each of the locations. At the end of each run, 0.5 cfu on average was found on the bottom left corner of the preparation area, near the parking area for vials and bags, and 0.17 cfu on average was found on the left side of the shaker. All results were within the limit of <1 cfu and thus met with grade A air criteria (Eudralex, GMP annex 1, 2008). The results of the settle plates are given in Table 2. Of the 36 incubated settle plates, 5 showed microbial growth. Of these, 4 came from the location in the loading area. For the compounding area, all results were within class A limits, but for the loading area, class A criteria were not met (average of 1.17 cfu).

Table 2. Results of the settle plate microbiological measurements inside the robot.

Number in Fig. 1	Location	Day 1 part 1 (cfu)	Day 1 part 2 (cfu)	Day 2 part 1 (cfu)	Day 2 part 2 (cfu)	Day 3 part 1 (cfu)	Day 3 part 2 (cfu)	Average (cfu)
18	Bottom right corner preparation area	-	-	-	-	-	-	0
19	Bottom left corner preparation area	-	-	-	-	-	-	0
20	Under the dosing device	-	-	-	-	-	-	0
21	Surface between preparation area and warehouse	-	-	-	-	1	-	0.17
22	Under the rotary in the warehouse	-	-	-	-	-	-	0
23	Right side of the loading area	-	1	4	1	-	1	1.17

Cfu = colony forming units

The results of the surface monitoring with contact plates are shown in Table 3. A total of 54 contact plates from 13 surface spots were collected. Of these, 5 showed microbial growth. On average, 12 of the sampled spots met class A criteria. Location 13, at the right side of the loading area, showed an average of 3 cfu, which does not comply with grade A.

Table 3. Results of the contact plate microbiological measurements inside the robot.

Number in Fig. 1	Location	Day 1 part 1 (cfu)	Day 1 part 2 (cfu)	Day 2 (cfu)	Day 3 (cfu)	Average (cfu)
5	Bottom right corner preparation area	-	-	-	-	0
6	Under the dosing device	-	-	-	-	0
7	Right side of the shaker	-	-	-	-	0
8	Left side of the shaker	1	-	-	-	0.25
9	Behind the shaker	-	-	-	-	0
10	Parking area for vials right side	-	-	-	-	0
11	Lateral surface of the preparation area	-	-	-	-	0
12	Left side of the loading area	1	-	-	-	0.25
13	Right side of the loading area	9	3	-	-	3
14	Lateral right side surface of the loading area	-	-	-	-	0
15	Lateral left side surface of the loading area	-	-	1	-	0.25
16	Left side of the rotary warehouse area	-	-	-	-	0
17	Right side of the rotary warehouse area	-	-	-	-	0

All cfu that were observed in both the air and the surface monitoring were common bacteria (staphylococcus and micrococcus species), no yeasts were found. From these results we now maintain the action level for microbiological monitoring for Grade A according to the GMP (action level=any result >1 cfu). Alert levels will be developed over time using the results of routine daily monitoring.

DISCUSSION

This validation study shows that the robot APOTECAchemo can perform individually compound parenteral solutions without risk of microbiological contamination. In addition, our robot can reuse vials repeatedly and safely. We conclude that automated compounding meets the microbiological requirements set in the European GMP for Grade A cleanroom environments. The loading area of the robot met Grade B (< 5 cfu)[12]. This area is separated from the Grade A compounding area by means of an air curtain. The background cleanroom in our hospital meets Grade C criteria. So the supplies are moved from Grade C to B in the loading area and then to Grade A in the compounding area, which gives a proper air sterility gradient. We will implement continuous microbiological monitoring as well as media fill simulations for infusion bags (using syringes to compound the bags, thereby thus also qualifying syringe compounding) as is mandatory under GMP.[12] Furthermore, we will implement periodical personal qualification of all technicians that have been trained to work with the robot (10 individuals as of January 2019).

In addition, this study shows that the robot can reuse vials repeatedly and safely, thereby enabling extended usage. Repeated puncturing of the vials of up to 6 times, and storage of these punctured vials up to 8 days was shown to be microbiologically safe. In daily clinical practice, not all reconstituted drugs are stable for this extended period, so in routine practice, for each individual drug, stability data are used to define the maximum reuse time. During unloading of the punctured vials, the robot provides a label with a unique barcode, which is recognized by the robot when the vial is reused. This workflow enables the reuse of vials of expensive cytostatics or monoclonal antibodies, without the risk of using degraded drug concentrates.

Our results are in line with several previous studies. A study by McLeod et al. on microbiological monitoring of a first generation robot has shown particles as well as microbiological cleanliness to meet GMP grade A limits in two separate hospital pharmacies, but media fill simulation tests were not performed.[16] Of the second generation robots, only data of APOTECAchemo are available. The first data of APOTECAchemo were collected and published in 2014, as part of a larger trial, investigating the performance of this robot in daily practice in an Italian hospital pharmacy.[17] Over several years, monthly testing was performed for sterility of compounded preparations in collaboration with the local microbiology laboratory without ever showing a contamination. However, this trial did not specify the number of samples and the manufacturing process for these sterility tests. A series of 108 media fill preparations in Denmark has shown more detailed data in abstract form, also demonstrating no bacterial growth.[8] By far the largest previous

study was performed in Mainz in Germany.[9] This study compared the manual preparation process with the robotic compounding using APOTECAchemo. One thousand media fill products were prepared in total, none of which demonstrated turbidity after incubation, thereby indicating no contamination with microorganisms. In addition, settle and contact plates of the compounding area met the grade A criteria as in our study. Also in Mainz, the loading area did not meet grade A, but contrary to our work, grade B was also not attainable for the loading area in their setting.[9] Our study confirms the work from the Mainz group and adds the possibility of reusing vials repeatedly and safely without microbiological risks. The only difference between microbiological monitoring results (Grade B for the loading area in our setting), could be a result of a cleaner background area in our pharmacy.

No previous studies have been published on repeated puncturing vials with a robot. One trial is available, where vials were left attached to a semi-automated filling device for compounding total parenteral nutrition. In this study, vials remained sterile for 24 h, but repeated puncturing or detaching and attaching to the machine was not addressed.[18]

Our present study validated the robot APOTECAchemo according to the European GMP and we are the first showing no contamination for repeated puncturing of vials. Hence, with our robot, preparing individual doses for multiple patients from one vial is microbiologically safe, even if these patients visit our oncology wards on different days. This can lead to major cost savings by saving vials of expensive cytostatic drugs and monoclonal antibodies.

A strong point of our study is that we performed this qualification on multiple days with rotating staff, thus simulating actual working protocols. Clearly, there are also limitations to the present study. Firstly, our study was performed in a single centre. Our cytotoxic compounding cleanroom is of class C. Hence, extrapolating our results to pharmacies with different cleanroom grades, i.e. grade D, cannot be done. Secondly, we used our maximum batch size of 32 preparations per session for the media fill simulation. Hospitals with a larger throughput should perform process validation in accordance with their local workload and protocols. Future studies are still warranted to repeat our investigation in settings where different disposables, different cleaning procedures or different classes of cleanrooms are used. Also media fill simulation tests of elastomeric pump devices with a robot remain to be executed.

In conclusion, robotic compounding of cytostatic drugs with APOTECAchemo meets the microbiological requirements of the European GMP. In addition, our robot can reuse vials repeatedly and safely, thereby enabling extended usage.

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