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Use of leftovers of monoclonal antibody products after partial extraction – A microbiological safety study

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Abstract

Background/purpose: In the absence of thorough microbiological, chemical and physical stability data, high amounts of pharmaceutical products, from which the seal has been broken, are to be discarded after preparation. We performed a generic microbiological validation study for several marketed monoclonal antibody products, in order to define conditions under which leftovers from partially extracted product can be used in order to minimize loss.

Methods: From the daily practice of the Central Preparation Unit of the Netherlands Cancer Institute, used monoclonal antibody product vials were collected. To examine the integrity of the primary packaging, a VDT/S Vacuum Leak tester from Erweka was used. Vials were punctured with different types of spikes or a needle prior to experiments and examined for leakage afterward. In addition, microbiological monitoring was performed by broth simulation of the preparation method.

Results: All vials (631 vials, 18 different monoclonal antibody products) showed no leakage after puncturing with a 18 G needle. However, the use of a spike system resulted in leakage in 108 of the 435 tested vials. Results from the broth simulations confirmed a higher risk of contamination after puncturing with a spike as compared to needle-punctured vials (0.5% vs. 0.05%).

Conclusion: When working under aseptic preparation conditions and making use of appropriate needle, the risk of contamination is acceptably low to justify storage and reuse of leftover monoclonal antibody product from a microbiological perspective. The spikes tested lead to an unacceptably high level of loss of integrity and subsequent risk of microbiological contamination if stored in a non-classified environment. We concluded that these results could be applied generically to all monoclonal antibody products with a primary packaging composed of a glass vial and rubber stopper.

Keywords

Microbiological safety, monoclonal antibodies, contamination, rubber stopper, needle

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Introduction

Monoclonal antibodies (mAbs) are used today for numerous purposes in scientific research, diagnostics and medical treatments. mAbs are complex biostructures and are produced by living organisms such as the Chinese Hamster Ovarian (CHO) cells.[1] mAbs bioengineered for various targets are available, both targeting oncological antigens, and other pathogenic antigens. The use of mAbs in the treatment of cancer has been successful, resulting in mAbs being the fastest growing class of new drugs approved for this

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indication.[2] All mAbs are administered parenterally. The product presentations of mAbs consist of a sterile concentrated liquid or a powder for reconstitution for dilution for infusion, packed in glass vials closed with a rubber stopper and cap. However, as dosing of many mAbs is based on body size and since the available dose unit strengths are limited, not always the full content(s) of the vial(s) is used for one preparation, resulting in a leftover product. The Summary of Product Characteristicts (SmPC) for each product instructs to discard these leftovers within 24 h after opening, reconstitution or preparation if not administered to the patient and do not provide additional chemical, physical and biological stability data. The general time frame of 24 h is mainly based on the risk of microbiological contamination.[3–20] Also, the Canadian National Association of Pharmacy Regulatory Authorities (NAPRA) and both the American United States Pharmacopeia (USP) and American Society of Health-System Pharmacists (ASHP) are stating that a single-use opened vial is to be used within 6 h.[21–24] The cumulative amount of these mAb leftovers may represent a significant loss of potentially active drug product, also from an economic perspective.

To reduce this loss, for some mAbs we have suggested fixed dosing, meaning that the same dose is given to every patient irrespective of body weight, resulting in the use of a distinct number of complete vials per preparation and a concomitant reduction in treatment costs.[25] To further reduce these costs, it is of interest to investigate whether residues in vials can still be used. Prerequisites for the use of leftovers are threefold: first, chemical and physical stability of the mAb of interest should be established and confirmed, second, retention of biological activity should be shown, and third, the risk of microbial contamination due to the previous handling should be minimal. The current study focuses on the reuse of these leftovers from a microbiological perspective.

As vial integrity is usually only tested on primary packaging prior to breaking the seal, integrity after the puncture is usually unknown. The research was carried out with product handled in daily hospital pharmacy practice, and to our knowledge, this makes the study novel.

Materials and methods

This study was carried out with mAb products from daily clinical practice at the Central Preparation Unit (CPU) of the hospital pharmacy of the Netherlands Cancer Institute (NKI). The pharmacy performs compounding of mAbs for approximately 15,000 preparations/year. Leftovers of compounding were used for the current study. The list of mAb products used in the experiments and characteristics (e.g. manufacturer, brand name, clinical indication, target, rubber material, etc.) can be found in Table 1. All mAbs were prepared aseptically according to the instructions given in the SmPC (see Figure 1). The penetration step that is performed by a needle or dispensing pin (spike) was performed in a Class A cabinet with laminar air flow (LAF) under Good Manufacturing Procedure (GMP) with a class D background.[26] Besides, continuous aseptic processing validation and monitoring of the environment to assure its classification were performed. A list and illustration of the tools for withdrawal of the product from the vials can be found in Table 2 and Figure 2, respectively. After preparation, the punctured vial was stored in a non-classified area.

Vacuum-leak test. Integrity testing of punctured vials was performed using a vacuum-leak test using a VDT/S Vacuum Leak Tester with a 300-mm diameter container (Erweka GmbH, Heusenstamm, Germany) (see Figure 3). In short, the principle of the test consisted of applying a vacuum in the exsiccator of the Vacuum Leak Tester, that is containing the punctured vials submersed in a methylene blue solution. A porcelain plate is covering the vials to guarantee they are below the surface of the solution during the whole experiment. Colouring solution will enter any vial with a breached integrity when releasing the vacuum, whereas intact vials will contain no coloring agent. For each vacuum-leak test run, 25–30 vials were processed depending on the vial size. The vacuum-leak test program was set to 400 mbar over 1 min. After each run, vials were collected, cleaned and visually examined for any blue colouration inside the vials (see Figure 4).

Screening experiment 1. Vials were collected during normal daily practice from the CPU after partial extraction of their content, this was conducted as a 'baseline' measurement for the current situation. During preparation, vials were penetrated with either a needle (BD Microlance^{TM} 3 18 G 1½″ 1.2 × 40 mm, Beckton, Dickinson, and Company) or a spike (CODAN Spike 4 mm diameter with 0.2 µm air-eliminating filter and female luer-lock with cap, CODAN, Medizinische Geräte GmbH & Co KG, Germany) by choice of the operator (see Figure 2). In some cases, the vials collected still had a spike inserted. Vials that did not contain a spike could have been either punctured with a needle or a spike, with the spike removed after the partial extraction. The brand Keytruda was always punctured with a needle in this experiment.

Screening experiment 2. Vials were collected from the CPU after partial extraction with either a spike or a needle (similar materials as used in screening experiment 1) by

Table 1. mAb products that were tested in the experiments.

mAbs	Product	Indication	Type and target	Manufacturer	Rubber stopper[a]	Presentation 1	Presentation 2
Atezolizumab	Tecentriq®	Urothelial carcinoma, non-small cell lung cancer (NSCLC)	IG1 anti-PD-L1 (programmed death-ligand 1 blocking)	Roch	Butyl rubber	1200 mg/20 mL	
Avelumab	Bavencio®	Merkel cell carcinoma (MCC)	IgG1 anti-PD-L1	Merck Serono Pfizer	Halobutyl rubber	200 mg/10 mL	
Bevacizumab	Avastin®	Metastatic carcinoma of n/rectum, metastatic BC, metastatic/advanced/recurrent NSCLC, RCC, Epiithelial ovarian, fallopian tube, primary peritoneal, cervix cancer	IgG1 anti-VEGF-A	Roche	Butyl rubber	100 mg/4 mL	400 mg/16 mL
Cetuximab	Erbitux®	EFGR-expressing RAS wild-type metastatic colorectal cancer, squamous cell cancer head/neck	IgG1 anti-EGFR	Merck Serono	Halobutyl rubber	100 mg/20 mL	500 mg/100 mL
Infliximab	Remsima®	Rheumatoid arthritis, psoriatic arthritis, rheumatoid colitis, ulcerative colitis, Crohn disease, psoriasis, spondylitis, ankylosing	IgG1 anti-TNF-alpha	Healthcare Celltrion	Butyl rubber	100 mg[b]	
Infliximab	Remicade®	Rheumatoid arthritis, psoriatic arthritis, rheumatoid colitis, ulcerative, Crohn disease, psoriasis, spondylitis, ankylosing	IgG1 anti-TNF-alpha	MSD	Rubber	100 mg[b]	
Ipililumab	Yervoy®	Melanoma	IgG1κ anti-CTLA-4	BMS	Coated butyl rubber	50 mg/10 mL	200 mg/40 mL
Nivlumab	Opdivo®	Melanoma, non-small cell lung cancer, renal cell carcinoma (RCC), classical Hodgkin lymphoma (cHL)	IgG4 anti-PD-1	BMS	Coated butyl rubber	40 mg/4 mL	100 mg/10 mL
Olaratumab	Lartruvo™	Soft tissue sacroma	IgG1 anti- PDGF-R alpha (platelet-derived growth factor receptor alpha)	Lilly	Rubber	190 mg/19 mL	500 mg/50 mL
Panitumumab	Vectibix®	Wilde-type RAS metastatic colorectal cancer (mCRC)	IgG2 anti-EGFR	Amgen	Elastomeric rubber	100 mg/5 mL	400 mg/20 mL
Pembrolizumab	Keytruda®	Melanoma, non-small cell lung cancer (NSCLC)	IgG4 anti-PD-1	MSD	Bromobutyl rubber	100 mg/4 mL	50 mg[b]
Pertuzumab	Perjeta®	Metastatic breast cancer, neoadjuvant treatment of BC	IgG1 anti-HER2	Roche	Butyl rubber	420 mg/14 mL	

(continued)

Table 1. Continued

mAbs	Product	Indication	Type and target	Manufacturer	Rubber stopper[a]	Presentation 1	Presentation 2
Ramucirumab	Cyramza®	Gastric cancer, gastro-oesophageal junction adenocarcinoma, metastatic colorectal cancer (mCRC), non-small cell lung cancer (NSCLC)	IgG1 anti-VEGF2	Lilly	Chlorobutyl rubber	100 mg/10 mL	500 mg/50 mL
Rituximab	Truxima®	Non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), rheumatoid arthritis, granulomatosis w. Polyangiitis and microscopic polyangiitis	IgG1 anti-CD20	Healthcare Celltrion	Butyl rubber	100 mg/10 mL	500 mg/50 mL
Rituximab	MabThera®	Non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), rheumatoid arthritis, granulomatosis w. Polyangiitis and microscopic polyangiitis	IgG1 anti-CD20	Roche	Butyl rubber	100 mg/10 mL	500 mg/50 mL
Tocilizumab	RoActemra®	Rheumatoid arthritis	IgG1 anti-IL-6 receptor (anti-interleukin-6)	Roche	Butyl rubber	200 mg/10 mL	400 mg/20 mL
Trastuzumab	Herceptin®	Breast cancer, gastric cancer	IgG1 anti-HER2	Roche	Rubber laminated with a fluoro-resin film	150 mg[b]	600 mg/5 mL
Vedolizumab	Entyvio®	Ulcerative colitis, Chron's disease	IgG1 anti-human $\alpha 4\beta 7$ integrin	Takeda	Rubber	300 mg[b]	

For all mAb products tested, the primary packaging material consists of a glass vial and a rubber stopper, sealed with an aluminum crimp cap and a plastic flip-off button.
[a]As stated in the SPC.
[b]Powder for reconstitution for dilution.

choice of the operator. For this experiment, the CPU was instructed to remove spikes immediately after the partial extraction. The preparation unit was also asked to mark the vials, so that needle punctured vials could be distinguished from spiked vials.

Spike experiment 3. Vials were collected from the CPU after partial extraction with either a needle (BD Microlance™ 3 18 G 1½″ 1.2 × 40 mm needle, Beckton, Dickinson, and Company) or different types of spikes as used in experiment 1 (CODAN MicroSpike Chemoprotect® 3 mm diameter with 0.2 µm air-eliminating filter and female luer-lock with cap, 5.0 µm fluid filter and female luer-lock with cap or a CODAN SWAN-LOCK® Spike 4 mm diameter, CODAN, Medizinische Geräte GmbH & Co KG, Germany) (see Figure 2). The MicroSpike was taken out directly while the SWAN-LOCK® spike was left in place. Choice of extraction method was chosen by the operator.

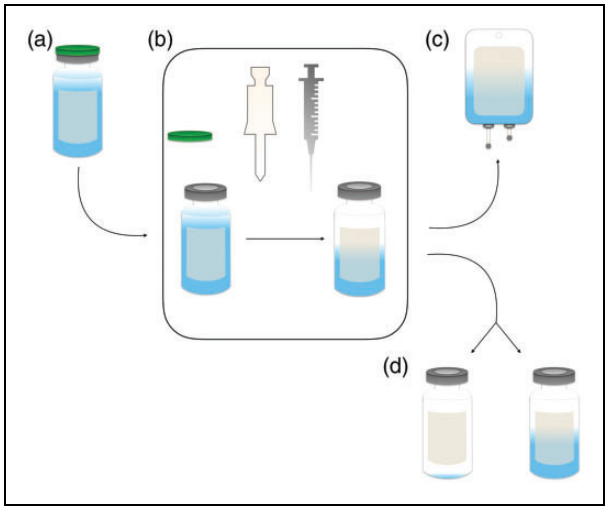

Figure 1. Flowchart of the preparation (a) concentrated mAb solution or powder for reconstitution for dilution, (b) operations performed in the Central Preparation Unit (CPU) in a Class A microbial safety cabinet with laminar airflow (LAF) and Class D background: Plastic flip-off button (green) is taken off. Either a spike or a needle is used to withdraw the concentrate from the vial (if needed, reconstitution of powder mAb is done before the withdrawal step) and diluted in infusion fluid in an IV bag. (c) A ready-to-use IV-bag to be administered to a patient. (d) On the left: a completely emptied vial to be discarded; on the right a vial with residual mAb concentrate.

Validation experiment 4. Vials that had only been punctured with a needle (BD Microlance™ 3 18 G 1½″ 1.2 × 40 mm needle, Beckton, Dickinson, and Company) were collected from the CPU and subjected to a vacuum-leak test. The specification used was extracted from the Dutch Hospital Pharmacist guideline: GMP-z (ziekenhuisfarmacie), Annex Z3 concerning process validation of aseptic compounding: the risk of contamination, in this case, translated to loss of integrity, is < 1% (95% confidence interval). In practice, this means that of 300 vials no loss of integrity is shown.[27]

Aseptic processing validation. Validation of aseptic handling is performed for each pharmacy technician for each working day by simulation of an aseptic preparation of an infusion solution using broth. From a vial containing broth (Tryptic Soy Bean, 10 mL, BioTrading Benelux, Mijdrecht, The Netherlands), 5 mL was extracted using a spike (CODAN Spike 4 mm diameter with 0.2 micron air-eliminating filter and female luer-lock with cap, CODAN, Medizinische Geräte GmbH & Co KG, Germany) and syringe; the spike was closed and left in the vial. Using a needle (BD Microlance™ 3 18 G 1½″ 1.2 × 40 mm needle, Beckton, Dickinson, and Company), the 5 mL of broth in the syringe is injected into a second vial of broth, and the needle is then removed. Both vials are incubated for a week at 37°C

Table 2. List of dispensing tool, their filter, lock, diameter, and manufacturer. For picture, see Figure 2.

Dispensing tool	Filter	Lock	Diameter	Manufacturer
CODAN Spike	0.2 µm air-eliminating filter	Female luer-lock with cap	4 mm	CODAN, Medizinische Geräte GmbH & Co KG, Germany
CODAN MicroSpike Chemoprotect	0.2 µm air-eliminating filter, 5.0 µm fluid filter	Female luer-lock with cap	3 mm	CODAN, Medizinische Geräte GmbH & Co KG, Germany
CODAN SWAN-LOCK® Spike	N/A	SWAN-LOCK®	4 mm	CODAN, Medizinische Geräte GmbH & Co KG, Germany
BD Microlance™ 3 18 G 1½″ 1.2 × 40-mm needle	N/A	N/A	1.2 mm	Beckton, Dickinson, and Company

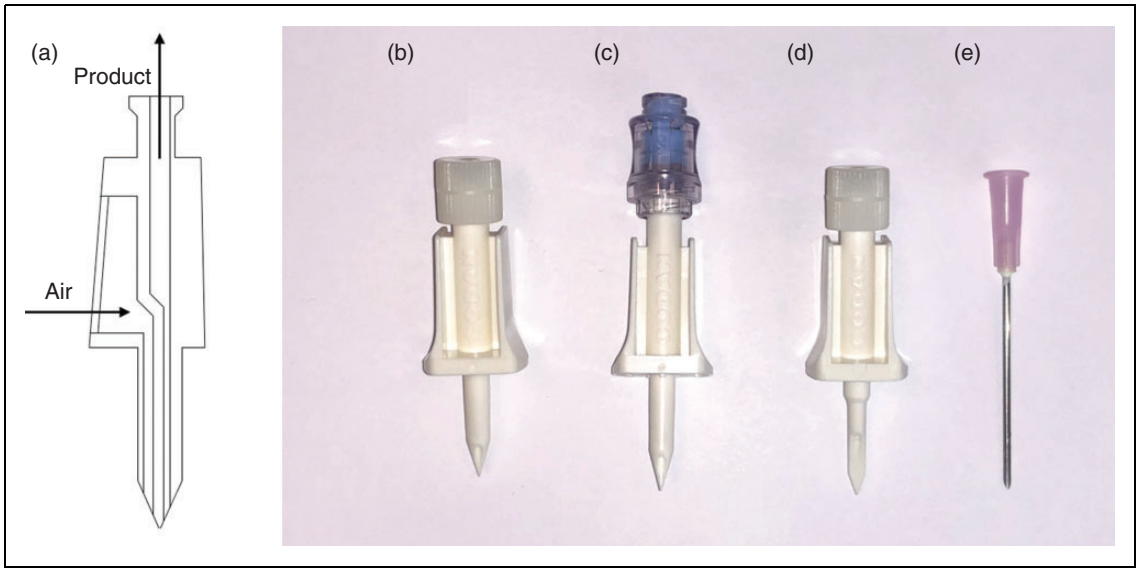

Figure 2. (a) Schematic picture of the inside of spike. The spike contains two channels: one starts from the top, where the syringe is attached, down to the pointed end, with which the rubber of the vial is penetrated. The other channel goes from the pointed end up to an air-eliminating filter making airflow into the vial possible without the risk of contamination. This prevents any vacuum or pressure building up inside the vial. (b) CODAN, (c) CODAN SWAN-LOCK®, (d) CODAN MicroSpike Chemoprotect®, (e) BD Microlance™ 3 18 G 1½″ 1.2 × 40-mm needle.

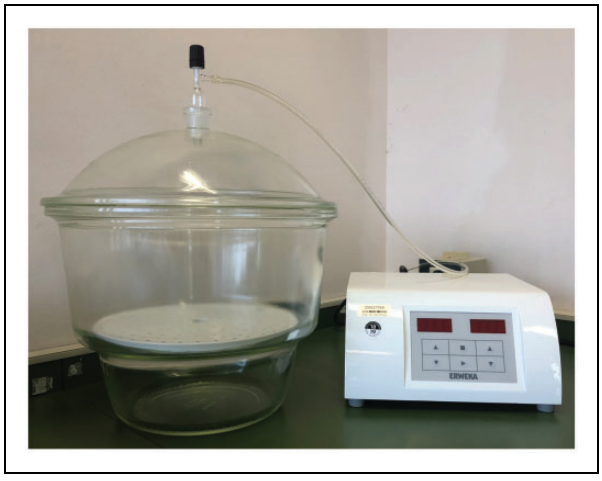

Figure 3. Picture of VDT/S vacuum leak tester with a 300-mm diameter container (Erweka GmbH, Heusenstamm, Germany).

and visually examined for turbidity. As specification the Dutch Hospital Pharmacist guideline: GMP-z (ziekenhuisfarmacie), Annex Z3 concerning process validation of aseptic compounding was applied: the risk of contamination is less than 1% (95% confidence interval). In practice, this means that of 300 simulations no contamination is shown.[27] The aseptic handling is continuously monitored, and results were collected from January 2017 until November 2018.

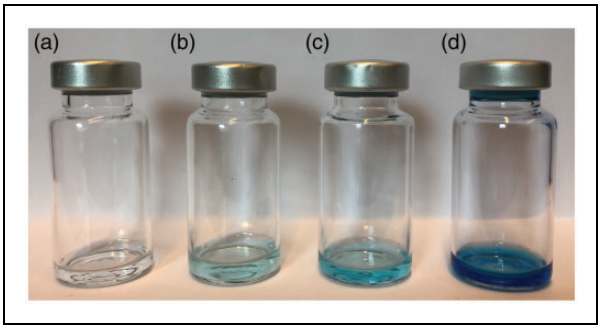

Figure 4. Picture of (a) negative vial. (b–d) Positive vials after leakage test in the VDT/S vacuum leak tester.

Results

Screening experiment 1. This baseline measurement included 518 vials from various mAb products that had been punctured with a needle or a spike, with the spike left in the vial or taken out. In total 96 (18.5%) of the vials tested positive for a leak. Of these only 23 (4.4%) were from the category with no spike left inside the vial. Full results are listed in Table 3.

Screening experiment 2. In total 143 vials were tested. The results showed that overall 17 (11.9%) vials showed positive results for a leak in the test. All of these vials were punctured with a spike. All vials

Table 3. Results from screening experiment.

Stopper material	Product	Spike	Total	Leak
Bromobutyl	Keytruda®	Yes	0	0
		No	69	0
Butyl rubber	Avastin®	Yes	24	7
		No	29	16
	Remsima®	Yes	19	1
		No	6	0
	Truxima®	Yes	15	10
		No	3	0
	Perjeta®	Yes	17	7
		No	0	0
	Tecentriq®	Yes	4	0
		No	0	0
	RoActema®	Yes	1	0
		No	1	0
	MabThera®	Yes	6	2
		No	12	1
Chlorobutyl	Cyramza®	Yes	2	0
		No	2	0
Coated butyl rubber	Opdivo®	Yes	80	14
		No	42	6
	Yervoy®	Yes	17	9
		No	5	0
Elastomeric	Vectibix®	Yes	6	3
		No	18	0
Halobutyl rubber	Erbitux®	Yes	30	10
		No	9	0
Rubber	Lartruvo™	Yes	18	8
		No	7	0
	Remicade®	Yes	1	0
		No	4	0
Rubber laminated with a fluoro-resin film	Herceptin®	Yes	6	2
		No	65	0
		Total	518	96
		% Leak		18.53

mAb products are clustered by stopper material (as extracted from the SmPC) and if they had a spike left in the vial (Yes/No), the total number of vials tested, and the number of vials with a leak. The total percentage of leakage was 18.5%, of this 4.4% of the vials without a spike showed leakage.

punctured with a needle did not show leakage in the test system. Full results are listed in Table 4.

Spike experiment 3. In total 153 vials were tested. Full results are listed in Table 5. The number of positive results for the spiked vials was 18 (11.8%). Leakage occurred only in vials punctured by a spike, irrespective of the type of spike used.

Table 4. Results from screening experiment.

Stopper material	Product	Spike (S)/ needle (N)	Total	Leak
Bromobutyl	Keytruda®	S	0	0
		N	24	0
Butyl rubber	Avastin®, Perjeta®, Remsima®, RoActema®	S	12	1
		N	15	0
Chlorobutyl	Cyramza®	S	0	0
		N	2	0
Coated butyl rubber	Opdivo®, Yervoy®	S	25	15
		N	13	0
Elastomeric	Vectibix®	S	0	0
		N	11	0
Halobutyl rubber	Bavencio®	S	1	0
		N	1	0
Rubber	Lartruvo™	S	2	1
		N	5	0
Rubber laminated with a fluoro-resin film	Herceptin®	S	12	0
		N	20	0
		Total	143	17
		% Leak		11.89

mAb products are clustered by stopper material (as extracted from the SmPC) and whether they were punctured with a needle or spike, the total number of vials tested, the number of vials with a leak. The total percentage of the leakage was 11,9%, of this 0% of the needle-punctured vials showed leakage.

Validation experiment 4. Final experiments included testing of 450 new vials that were all punctured with a needle. Together with the previous results, this added up to a total of 631 vials. There were no positive results found in the leak test of these vials. Results are listed in Table 6, grouped in rubber material, brand, number of vials from the validation and the total number of needle-punctured vials tested throughout all experiments.

Broth simulation. Collected results from the years 2017 and 2018 can be seen in Table 7. Contamination occurred in 0.5% (9 out of 1691 tested simulations) in the spiked vials and 0.05% (1 out of 1873 simulations) in the needle-punctured vials.

Discussion

Microbiological contamination of a mAb vial can occur either during preparation or at a later stage when the

Table 5. Results from experiment.

Stopper material	Brand	MicroSpike (MS)/ SWAN-LOCK Spike (SS)/Needle (N)	Total	Leak
Bromobutyl	Keytruda®	MS	3	0
		N	0	0
Butyl rubber	Avastin®, Perjeta®, Remsima®, RoActema®, Truxima®	MS	16	2
		SS	6	2
		N	7	0
Coated butyl rubber	Opdivo®, Yervoy®	MS	84	8
		SS	7	5
		N	2	0
Halobutyl rubber	Bavencio®, Erbitux®	MS	2	0
		SS	4	0
		N	3	0
Rubber	Lartruvo™, Remicade®, Entyvio®	MS	12	0
		SS	1	0
		N	2	0
Rubber laminated with a fluoro-resin film	Herceptin®	MS	1	0
		SS	1	1
		N	2	0
		Total	153	18
		% Leak		11.8

mAb products are clustered by stopper material (as extracted from the SmPC) and whether they were punctured with a MicroSpike, a SWAN-LOCK spike or needle, the total number of vials tested, and the number of vials with a leak. The total percentage of the leakage was 11.8%, of this 0% of the needle-punctured vials showed leakage.

Table 6. Total number of needle-punctured vials clustered by stopper material and mAb product.

Stopper material	Brand	Total
Bromobutyl	Keytruda®	302
Butyl rubber	Avastin®, Perjeta®, Remsima®, RoActema®, Truxima®	74
Chlorobutyl rubber	Cyramza®	2
Coated butyl rubber	Opdivo®, Yervoy®	95
Elastomeric	Vectibix®	21
Halobutyl rubber	Bavencio®, Erbitux®	7
Rubber	Lartruvo™, Remicade®, Entyvio®	23
Rubber laminated with a fluoro-resin film	Herceptin®	107
	Total	631

partially emptied vial is stored. In all cases, contamination will be due to microbiological ingress as a consequence of breached vial-stopper integrity or inadequate aseptic processing.

In our CPU, the content of a vial was extracted by penetrating the rubber stopper by either a needle or a spike connected to a syringe. Compared to a needle, a spike makes the withdrawal of the content easier for the operator. Besides a larger diameter, a spike has two canals, one for withdrawal of the content in the vial and another to let air pass down to the vial through a filter. The filtered air canal results in no vacuum or

Table 7. Results from broth simulations in January 2017–November 2018.

Preparation of vial	*n*	*n* contaminated	% contaminated
Spiked	1691	9	0.53
Needle punctured	1873	1	0.05

pressure building up in the vial while withdrawing its content (see Figure 2). On the other hand, the spike makes a bigger hole in the rubber stopper during puncturing than needles.

In the CPU of the NKI, the working environment for the aseptic preparations is a Class A microbiological safety cabinet with an LAF and a Class D background.[26] Storage of partially extracted vials in the Class A flow cabinet would prevent microbial contamination but will consume preparation space. Moreover, line clearance of the cabinet before a next preparation is not possible with an increased risk of product mix-up. Preferable, used vials need to be stored in a non-classified environment before reuse.

To examine the possibility of using residual mAbs from a microbiological contamination perspective, we used two methods. First, we screened the vial-stopper integrity of mAb products after initial use (i.e. puncture with needle or spike) in the current situation, followed by experiments to examine possible improvements. Secondly, we collected and reviewed data from the continuous aseptic processing validation program. Other studies have studied if the content of vials is contaminated after handling by adding them to a broth solution and then perform visual examination.[28,29]

From the first screening experiment, it was shown that the current procedure was inadequate to ensure the integrity of used vials. Reuse of leftovers requires 100% integrity of all mAb products used at the CPU to assure sterility. As significantly fewer vials showed leakage after removal of the spike or when they were only needle-punctured (18.4% vs. 4.4%), the second experiment was focused on the use of needles and the removal of the spike after puncturing. This experiment clearly showed that puncturing with a spike and its subsequent removal led to the inadequate closure of the vial. However, needle-punctured vials did not show any leakage. This is probably due to the smaller diameter (1.2 mm) of the needle as compared to that of the diameter of the spike (4 mm) used in this experiment. In case of the needle puncture, the stopper is undergoing less mechanical impact and seems capable of complete re-closure after retraction, irrespective of the stopper material.

As spikes are more convenient for the operator, two alternative spikes were tested. These were the MicroSpike that has a smaller diameter and the SWANLOCK® spike that has a different closure-system but the same diameter as the normal spike. With this MicroSpike leaks occurred less frequently compared to the SWAN-LOCK® spike (9.5% compared to 11.9%), however, these results are not sufficient for fulfilling the criteria set for the integrity. On the basis of these results, it was concluded that only needle-punctured vials had remained intact and are a candidate for reuse. To validate this, the test set of needle-punctured vials was increased to 631, and it was shown that all were intact which complied to the set specification of less than 1% loss of integrity (95% confidence interval).

The results were grouped by rubber stopper material as this is considered to be the critical aspect of the integrity of the primary packaging material of the mAb products. The factors taken into account were the glass vials, rubber material, the shape and diameter of the rubber stopper, the way the product is stored. Today the glass vials are made from glass tubings compared to the old molding process. The result is a glass vial with less cosmetic defects; better dimensional consistency.[30] The most critical part of the packaging is the rubber. All the stoppers have a complex chemical rubber formulation in the form of an elastomer, which has excellent characteristics for being used as a stopper for glass vials containing pharmaceuticals.[31] Some rubbers are laminated with different coatings or have a halogen in their chemical structure. The coating is done to reduce the leaching of rubber components into the drug product.[32] The mechanical tension on the rubber might be altered for the stoppers that were laminated. This could lead to easier conformation to its original form after being punched, as seen in the results of Herceptin®. The rubber comes in two designs; the serum rubber stopper and the lyophilization rubber stopper.[33]

No difference in performance after puncture could be shown for the different types in the current study. As a final consideration, the storage conditions were taken into account. All vials were stored without UV exposure at refrigerated temperatures $5 \pm 3^{\circ}C$, according to the SmPCs.[3–20] The vials were all used before the expiry date, which means that the rubber should be of adequate quality. Properties of the rubber were evaluated without any definite conclusions why there were leaks when using spikes. However, conclusions were made that all the different rubber stoppers can withstand a needle punch without losing their integrity. After looking at the results from the different products, we can conclude that the issues and solution are generic. If no dramatic change occurs in the manufacturing process of glass vials with a rubber stopper, the conclusion can be applied to other brands as well.

Broth simulation. The broth simulation of the preparation method was included in this study to complement

the results. These experiments were performed using the normal spike, where the spike was left in the vial, or by using a needle and removing it after puncturing.

The levels of contamination found in this broth simulation are well within the criteria set for contamination confirming adequate aseptic processing at the CPU.[27] Indeed, a higher level of contamination is seen for the spike-punctured vials, which is a confirmation of the integrity test results, which show that the use of a needle is more reliable from a microbiological contamination perspective (Table 7).

Conclusion

When working under aseptic preparation conditions and making use of an appropriate needle, the risk of contamination is acceptably low to justify storage and reuse of leftover mAb product from a microbiological perspective. The spikes tested lead to an unacceptably high level of loss of integrity and subsequent risk of microbiological contamination if stored in a non-classified environment. We concluded that these results could be applied generically to all mAb products with a primary packaging composed of a glass vial and rubber stopper. In addition, the chemical/physical and biological stability needs to be examined before approving the re-use of leftover mAb product.

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