Evaluating Sterility of Single Dose Vials on an Automated Compounding Device

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ABSTRACT

Background: Current guidelines for sterile compounding require that single dose vials of pharmaceuticals must be discarded after 6 hours when accessed in an ISO Class 5 environment. At this time, no studies have evaluated the sterility of single dose vials at any time after opening.

Objective: The purpose of this study is to evaluate the sterility of single dose vials attached to an automated compounding device for up to 24 hours and accessed and maintained within a cleanroom environment.

Methods: This is a prospective, observational study evaluating the sterility of 32 pooled samples of manufactured single dose injectable drugs attached to an automated compounding device for up to 24 hours and maintained within an ISO Class 5 environment in an ISO Class 7 buffer area. Each pooled sample was comprised of the remaining contents of 10 single dose vial additives that were used for total parenteral nutrition (TPN) and attached to the compounder within the previous 24 hours. Samples were evaluated using membrane filtration sterility testing and incubated for 14 days per USP <71> requirements.

Results: The results revealed zero failed sterility samples. Single dose vials remained attached to the compounder for an average of 23.8 hours (± 0.1 hours). The average volume per sample was 879 mL (± 105.1 mL). Manipulation of vials during the compounding process included an average of 20.4 manipulations (± 1.4).

Conclusions: Single dose injectable drugs attached to an automated compounding device within an ISO Class 5 cleanroom environment may remain sterile for up to 24 hours. Future studies are needed with a larger sample size and under continued dynamic working conditions to provide further evidence to extend the beyond use date within USP <797>.

Key Words—single dose vials, sterile compound; sterility testing, USP <797>; TPN; USP <71>

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The U.S. Pharmacopeia Chapter 797 Guidebook to Pharmaceutical Compounding—Sterile Preparations (referred to as USP <797>) was published as a legally enforceable guideline in 2007 to govern the practice of sterile compounding. Its purpose is to standardize institutional practice in relation to air quality, environmental controls, personnel training, and beyond use dating. USP <797> establishes the beyond use date for manufactured vials that have been opened or accessed during the compounding process. The beyond use date is defined as “the date or time after which a product should

not be stored or transported” and is determined by the type of manufactured vial and the environment in which the vial is accessed. Manufactured single dose vials do not contain preservatives to protect against microbial growth and are given a beyond use date of 6 hours by USP <797> when accessed within an ISO Class 5 environment that is also contained within an ISO Class 7 buffer area. ISO Class 5 air quality is required in the direct compounding area and must contain less than 3,520 particles per cubic meter. USP assigned this beyond use date to single dose vials based on the assumption of risk that

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microbes are introduced when the seal is punctured and the expected microbial growth that occurs after exposure. According to the 2016 USP <797> Compliance Survey, 95% of sterile compounding facilities reported compliance with sterility requirements for single and multiple dose vials.¹

Single dose vials are frequently used with an automated compounding device during the preparation of parenteral nutrition for patients requiring intravenous feeding. In the 2014 ASHP National Survey of Pharmacy Practice, more than half of hospitals with over 400 beds utilized an automated compounding device to prepare parenteral nutrition.² Depending on the manufacturer and model of the automated compounding device, up to 24 additives may be attached to allow the addition of numerous small volumes into the solution. Single dose vials attached to the compounding device must be used within 6 hours after accessing and any remaining quantity of drug in the vial must be discarded at that time. This translates to numerous vials that are wasted each day when compounding continuously. Most of the single dose vial additives used in the parenteral nutrition compounding process have experienced drug shortages of varying severity over the past year. To manage shortages, facilities have had to ration limited supplies and patients have had to go without select nutrients for short periods of time. **Table 1** lists common parenteral nutrition additives used at institutions preparing products with an automated compounding device. Extending the beyond use date of single dose vials attached to an automated compounding device from 6 hours to 24 hours could help manage shortages and reduce waste of these products.

Care must be taken when extending the beyond use date for single dose vials used for compounding because of the potential risk for microbial contamination and subsequent patient harm related to infection. According to USP <797>, parenteral nutrition is considered a medium risk preparation because the number of manipulations that occur as attaching or removing sterile vials from the compounder can increase the risk of contamination.³ Additionally, available nutrients within the solution may support microbial growth should contamination occur. Recent reports of infections related to compounded parenteral nutrition products emphasize the importance of rigorous environmental controls and personnel trained in compliance with compounding guidelines.^{4,5} Any extension of beyond use dating that

Table 1. Examples of single dose vial additives included in sterile compounding procedures utilizing an automated compounding device for parenteral nutrition

	Additive	Vial size	Manufacturer
1	Ascorbic acid 500 mg/mL	50 mL	Mylan
2	Calcium gluconate 0.485 mEq/mL	50 mL	Fresenius Kabi
3	Famotidine 10 mg/mL	20 mL	Westward
4	Infuvite Adult ^a	100 mL	Baxter
5	Magnesium sulfate 4 mEq/mL	20 mL	Hospira
6	Potassium acetate 2 mEq/mL	20 mL	Hospira
7	Potassium chloride 2 mEq/mL	250 mL	BBraun
8	Potassium phosphate 3 mEq/mL	50 mL	APP
9	Sodium acetate 2 mEq/mL	100 mL	Hospira
10	Sodium chloride 23.4%	100 mL	APP

^aInfuvite is available as 2 (50 mL) vials that were mixed prior to attachment and are stable for up to 4 hours after mixing.

differs from USP <797> requires analysis of sterility data to ensure that the new practice will not increase the risk of contamination.

Guidelines for sterility testing to extend the beyond use date for any sterile compound are outlined in USP Chapter 71–Sterility Testing.⁶ Two methods of sterility testing are discussed: membrane filtration and direct inoculation. Sterility testing should include growth media for aerobic, anaerobic, and fungal microorganisms. To achieve satisfactory results to support extension of the beyond use date for the compounded product, no contamination can be present in any of the samples collected.

At this time, there have been no studies published on the sterility of single dose vials accessed and maintained in an ISO Class 5 cleanroom environment. Two studies have examined the sterility of other products used in parenteral nutrition compounding, including manufactured intravenous fat emulsion solutions repackaged into smaller volume syringes for neonatal use. Dr. Catherine M. Crill and associates, in 2010, found that repackaging IV fat emulsion into sterile syringes resulted in a 3.3% contamination rate. However, samples obtained from manufactured products that were decreased in volume using a draw down method did not result in contamination after 24 hours.⁷ The draw down method used in the study resembles

vials attached to an automated compounding device. In a similar study conducted in 2002, sterility of IV fat emulsion in plastic syringes had a contamination rate of 3.3% at 7 days when repackaged with aseptic technique under a horizontal laminar airflow hood in a positive airflow room.⁸ None of the studies evaluated sterility of products that were accessed and maintained in an ISO Class 5 environment. The current study is needed to evaluate the sterility of single dose vials attached to automated compounding equipment for up to 24 hours in an ISO 5 cleanroom environment as a first step to extend their beyond use date.

This study was conducted at a 956-bed, academic medical center with an off-site sterile compounding facility. The sterile compounding facility provides batch compounding of selected products and patient-specific daily parenteral nutrition products utilizing several automated compounding devices maintained in an ISO Class 5 environment within an ISO Class 7 buffer room. Sterility testing is routinely conducted for batch compounds in compliance with USP <797> and USP <71> requirements. The medical center prepares almost 8,000 parenteral nutrition products per year for adult, pediatric, and neonatal inpatients. To meet compliance for beyond use dates of single dose vial additives, parenteral nutrition is compounded at the institution for only 6 hours every day and orders received outside this compounding window are processed on the following day. The study was conducted by the pharmacy team trained in USP <797> compliance during October 2015 through February 2016. The sterile compounding facility was certified in USP <797> environmental and procedural compliance throughout the study period.

OBJECTIVE

The purpose of this study is to evaluate the sterility of single dose vials attached to an automated compounding device when accessed and maintained within a cleanroom environment. Demonstrating sterility at approximately 24 hours is an initial step in lengthening the beyond use date for vials attached to automated compounding equipment. This could lead to potential savings for hospitals and compounding facilities working to manage drug shortages related to manufactured pharmaceutical products.

METHODS

This is a prospective, observational analysis of the sterility of 32 pooled samples of single dose vial additives obtained after attachment to a Baxter

EM 2400 automated compounding device for up to 24 hours. Pooled samples were collected to more closely resemble parenteral nutrition compounding processes and allow adequate volume for membrane filtration sterility testing. The same 10 single dose vial additives used at the institution were included in each sample and are listed in **Table 1**.

To minimize variation, samples were collected on days when the study-trained pharmacy technician was scheduled to prepare parenteral nutrition (approximately 3-4 days per week). A goal of at least 30 samples was considered reasonable within the study time period. Forty samples were initially collected and analyzed, with zero failed sterility samples. However, 8 samples were collected about 4 hours early due to required weekly cleaning of the cleanroom. These samples were excluded from the final analysis because the single dose vials were not allowed to remain attached for up to 24 hours.

Sampling for sterility testing occurred around the compounding schedule established at the facility. At the same time each day, the automated compounder was cleaned and a new vial set attached. Vials were scanned at the time of attachment to the Baxter EM 2400, which provided a complete report listing the lot number, manufacturer expiration date, and time of access for each vial.⁹ The time of initial set up was used to calculate the maximum time that vials remained attached to the compounder.

After setting up the compounder, parenteral nutrition was compounded for 6 hours to maintain compliance with current USP <797> guidelines for single dose vials. A daily median of 10 adult parenteral nutrition products were prepared on the designated compounder during the study period. When compounding, vials were replaced as needed to meet the demand for individual parenteral nutrition products ordered that day. Each exchange of a single dose vial was recorded as a manipulation. The minimum exposure time was calculated from the time that the last vial was exchanged until sample collection on the following day.

After compounding the daily parenteral nutrition products for up to 6 hours, the compounder and vials were allowed to remain attached overnight with no further manipulation. The time elapsed between the last parenteral nutrition product compounded and sample collection was roughly 18 to 22 hours. The following day, immediately prior to setting up the compounder again and approximately 24 hours after initial set up, a pooled sample was collected of all the vials attached. Samples were collected by draining the

contents of all the single dose vials into a Baxter EVA 2000 mL sterile empty container, with up to 1,200 mL of sterile water added to each sample to prevent precipitate formation between the calcium and phosphate additives.

Sterility testing of the pooled sample was conducted immediately after collection using membrane filtration sterility testing.¹⁰ This method was preferred over direct inoculation for the following reasons. Membrane filtration sterility testing allows the majority of each sample to be tested, while direct inoculation tests only a small portion of each sample. The risk for false positives is lower with membrane filtration sterility testing, and the sensitivity is higher when compared to direct inoculation.¹¹ In addition, salt concentrations of some additives included in each sample may have inhibited microbial growth using other sterility test methods.

The pooled sample was first accessed with a Steritest unit tubing set and then transferred in equal amounts through 2 Steritest EZ filtration canisters that contained 0.45-micron filters to retain microorganisms. A Steritest Equinox pump was used to transfer the sample at a controlled rate through the filtration canisters. After filtering the sample, Millipore Rinse Fluid A was pumped through the filtration canisters to remove any traces of the sample that may contain antimicrobial properties. Fluid Thioglycollate Media (FTM) was added to one canister to facilitate anaerobic microbial growth, and Soy Casein Broth (SCB) was added to the other canister to facilitate aerobic and fungal microbial growth. The canisters were labeled by date collected and incubated for 14 days at the appropriate temperature (SCB at controlled room temperature 20°C to 25°C, and FTM at 30°C to 35°C).

Every day, the sterility samples were assessed for evidence of contamination by the designated inspector at the compounding facility. If the media in the canister was turbid (cloudy) or there was particulate matter visible at any time during the 14 days, the sample was considered contaminated and recorded as a “fail” on the sterility test log. To reduce the risk of bias, the samples were labeled with standard batch numbers and stored with other batch testing being conducted at the facility during the same time period.

Observable data for each sample were recorded and stored in a labeled plastic binder near the compounding area to be completed by the pharmacy technician/investigator each day. A report of each

Table 2. Total time single dose vials were attached to the automated compounding device

Sample	Max. exposure time (hr:min)	Min. exposure time (hr:min)
1	23:45	21:31
2	23:30	21:28
3	23:35	22:00
4	23:45	21:25
5	24:15	21:18
6	24:00	21:11
7	23:50	21:26
8	23:45	21:54
9	23:40	22:00
10	24:00	22:34
11	24:08	22:01
12	23:55	21:50
13	23:30	21:15
14	24:15	23:13
15	24:15	21:10
16	23:35	22:09
17	23:45	21:35
18	23:35	21:21
19	24:05	21:40
20	23:55	22:01
21	23:55	22:25
22	23:55	22:35
23	23:36	21:31
24	23:40	22:05
25	23:25	21:48
26	23:23	21:00
27	23:45	21:10
28	24:20	22:18
29	22:55	21:20
30	23:55	21:48
31	23:15	21:51
32	23:50	21:15
Average, hours	23.77	21.75
95% CI	0.10	0.17

Note: Single dose vials remained attached to the automated compounding device for up to 24 hours. Maximum exposure time occurred from the time of initial set-up until the sample was collected, and minimum exposure time occurred from the time the last vial was exchanged during the 6-hour compounding process until sample collection.

product used during the compounding day was recorded that included the manufacturer, lot number, and expiration date of each ingredient sampled. Sterility testing data were recorded using the facility's sterility test log form. After the samples were collected and evaluated, the data were transferred to Microsoft Excel for further data analysis.

Data analysis included basic descriptive statistics, including mean and standard deviation with confidence intervals for continuous variables or proportions and frequencies for categorical data. Continuous variables were grouped and converted to categorical variables for data analysis. Rates of contamination were reported as a percentage of the samples that were contaminated out of 32 total samples.

RESULTS

The results of this study revealed zero failed sterility samples. Single dose vial additives remained attached to the compounder for a mean of 23.8 hours (± 0.1 hours). During the 6-hour compounding window, vials were exchanged as needed. The minimum exposure time for any vials exchanged during compounding was an average of 21.8 hours (± 0.17 hours). Infuvite was included in the sterility analysis,

although the manufacturer recommends the product be used within 4 hours due to chemical instability. Table 2 indicates the time that vials remained attached to the compounder.

The average volume of each sample was 879 mL (± 105.1 mL) and included the remaining contents of each additive attached to the compounder plus a sufficient volume of sterile water for injection to prevent precipitate formation between the calcium and phosphorous additives. There were 4 samples (10%) that developed precipitate formation within the filtration canisters after membrane filtration sterility testing. Exact causes of the precipitate formation are not clear at this time. Since the precipitate was not determined to be a microbial contaminant, the samples were included in the analysis. Figure 1 provides the average volume of each sample collected.

The average number of manipulations was calculated by recording the vials exchanged on the compounder during the 6-hour compounding day. On average, 20 vials (± 1.4 vials) were changed each day, depending on the volume of TPN products compounded. Figure 2 provides the total number of manipulations for each sample. Calcium gluconate was replaced an average of 5 times during

Figure 1. The total volume of each sample varied based on daily demand of additives and the quantity of water needed to prevent precipitate formation between calcium and phosphate additives included in the sample. A daily median of 10 adult parenteral nutrition products was prepared during the study on the designated compounder. Total volume trended downward as the study progressed due to lower volumes of phosphate included in later samples and less need for additional water.

Figure 2. Total manipulations were recorded as the number of vials changed on the automated compounding device during daily par-enteral nutrition compounding and represent an increased risk of touch contamination within each sample.

compounding, whereas ascorbic acid and potassium chloride did not require replacement. The number of times a vial was replaced correlated with the size of the vial and patterns of use within the parenteral nutrition products, with larger vials requiring less frequent exchange. Figure 3 shows the average number of manipulations associated with each additive. Between 4 and 20 adult parenteral nutrition products were prepared each day during the study, with a median of 10 products per day.

The average volume of each additive included in the samples varied according to daily usage and is shown in Figure 4. Potassium chloride was included in the highest quantities for each sample with an

average volume of 109.1 mL (± 15.1 mL). This is likely related to the size of the vial (250 mL) that was used during the study. Magnesium sulfate was included in the smallest quantity for each sample, with an average of 8.9 mL (± 2.2 mL), and originally contained in a 20 mL vial.

DISCUSSION

The results of this study demonstrate that the sterility of single dose vials may extend beyond 6 hours, and possibly up to 24 hours, when attached to an automated compounder and accessed and maintained within a cleanroom environment. In an effort to reduce costs associated with pharmaceutical waste

Figure 3. Additives were changed during parenteral nutrition compounding as needed, with smaller vials with higher demand tending to require more frequent exchange.

Figure 4. The volume of additive varied in each sample based on the vial size, daily demand, and remaining contents after compounding. Vial sizes for additives varied from 20 mL to 250 mL.

and manage ongoing drug shortages, this represents a first step in extending the beyond use date for single dose vials. Future studies are needed with a larger sample size and under continued dynamic working conditions to provide further evidence to extend the beyond use date within USP <797>.

Setting up the automated compounder for parenteral nutrition with the same 10 single dose vials in the study costs approximately \$210 using wholesale acquisition pricing. In a 24-hour compounding facility, exchanging the vials every 6 hours per USP <797> would cost an additional \$230,000 per year.

Strengths of the study included the use of a pooled sample to more closely resemble parenteral nutrition compounding and exchanging vials on the compounder throughout the 6-hour compounding period to allow for dynamic working conditions. Both imparted a higher risk of contamination that was acceptable in the evaluation of sterility. An additional strength of the study was the use of membrane filtration sterility testing to analyze the entire contents of the sample for sterility, as outlined in USP <71> Guidelines for Sterility Testing.

A potential limitation included the use of a single trained technician for compounding parenteral nutrition and conducting sterility testing. This was chosen to decrease variation in adherence to the research protocol. However, multiple personnel would routinely be involved in preparation of parenteral nutrition, increasing the risk of touch contamination. Another limitation occurred with the vials remaining attached for up to 18 hours in static conditions without interference. In 24-hour compounding facilities,

the vials would be exchanged at all hours, increasing the number of manipulations and the risk of contamination.

During evaluation of the data, it was identified that the famotidine included in the samples was a multiple dose vial that contained the preservative benzyl alcohol. Famotidine is no longer available in single dose vials due to shortages. Analysis of the volume added to each sample indicated that the concentration of benzyl alcohol ranged from 0.0% to 0.02% in the samples, with an average of 0.009%. At this concentration, according to Karabit et al, it would take between 167 and 10,970 hours to inhibit microbial growth of most microorganisms.¹² The pooled samples were filtered within 15 minutes of collection, and the filters were rinsed to remove any antimicrobial properties of the solution at that time. The study investigators deemed that this trace amount of preservative was not likely to have impacted the sterility results of the study.

Interestingly, there were 4 samples (10%) that formed precipitate within the filtration canisters after the membrane filtration sterility test. The precipitate was white to gray in color and appeared as 5 to 10 mm flakes that rapidly settled within the FTM or soy casein broth. No turbidity or alteration in the clarity of the solution was noted. It is difficult to determine which additives formed the precipitate at this time.

Further studies are needed to validate the extension of sterility for single dose vials when accessed and maintained within a cleanroom environment and not attached to an automated compounding device.

Currently, batch testing is required on any product assigned a beyond use date outside of USP <797> recommendations.

CONCLUSION

The results of this study may be used to justify additional research to focus on extending the current beyond use date for single dose vials from 6 to 24 hours. This could lead to reduction of product waste and improved management of drug shortages and contribute to significant cost-savings for health care facilities.

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