
A 7 Days Microbial Ingress Test of Single Use Vials Utilizing the EQUASHIELD[®] Closed System Drug Transfer Device

Study by Nelson Laboratories (Salt Lake City, UT) 2013, updated in 2014*

Study Summary

Abstract:

Aim

To perform an extreme-use-conditions test and assess the ability of the Equashield[®] Closed System Transfer Device to prevent the microbial contamination of parenteral drug vials during 7 days of use.

Method

Four groups of vials containing growth media were accessed with 770 Equashield[®] syringes. Each vial was accessed 10, 7 and 5 times respectively during 7 days using the Equashield[®] CSTD and 4ml were drawn off each time.

Before each of the 770 accesses, the access membranes of Equashield[®] Vial Adaptors were inoculated with ($>10^3$ CFU) with gram positive or gram negative bacteria respectively and were left to settle during $\frac{1}{2}$ an hour on the membrane. Initially the Vial Adaptors were spiked onto the media-filled vials two days earlier and incubated, resulting in a total of 9 days of testing, from first spiking to last access, and a total of 770 tested Equashield[®] Syringes and vial accesses.

The tested vials were incubated once again after the 9 days of testing and examined to demonstrate the ability of the Equashield[®] CSTD to prevent vial contamination during 7 days of use. To further increase the challenge and the extreme use conditions, approximately a third of the tested Equashield[®] devices were old expired products.

Results

All tested media-filled vials were negative for growth of any microorganisms.

Introduction:

Equashield[®] is a Closed System Transfer Device (CSTD) for the preparation and administration of hazardous drugs and it is cleared under FDA's product code ONB for "closed antineoplastic and hazardous drug reconstitution and transfer systems". It complies with NIOSH's definition: "A drug transfer device that mechanically prohibits the transfer of environmental contaminants into the system and the escape of hazardous drug or vapor concentrations outside the system"².

As a CSTD that is designated for working with hazardous drugs, the compliance of Equashield® with especially the second part of NIOSH's definition² has been extensively tested in clinical and scientific studies and proven to be compliant, namely: "mechanically prohibits the escape of hazardous drug or vapor concentrations outside the system". In order to achieve full closure of the Equashield® CSTD and contamination free connections, unprecedented mechanisms and design features were incorporated into the CSTD, which in turn created a natural advantage for the CSTD over conventional systems and methods, whereby the risk of the transfer of microbial contaminants into the system is minimized.

In another aspect, the United States Pharmacopeia (USP) Chapter 797 standards¹ mandate that single-use or non-preserved drugs must be discarded 6 hours after first vial access, if the vial is accessed and remains in ISO class 5 air conditions, otherwise it must be discarded after 1 hour. The purpose of this USP standard¹ is to provide patient protection by limiting the impact potential of any microbial contamination of the drug. On the other hand, it creates significant waste of partially used viable drugs, which in turn, increase financial expenditure. This includes the high-cost drugs which are discarded in spite of the fact that the majority of them remain chemically stable for days and weeks.

Published economical surveys demonstrate the obvious saving potential of the wasted drugs (in \$Millions) if CSTDs can be proven capable of prohibiting the transfer of microbial contaminants into the drug vial to maximize the utilization of the drug. Furthermore, such savings can also finance the cost of the CSTDs.

The Phaseal® CSTD (Becton & Dickinson) has demonstrated the potential to prevent microbial ingress in vials up to 7 days in the following studies: "Utility of the PhaSeal Closed System Drug Transfer Device", Derek M. McMichael et al., *Am J Pharm Benefits*. 2011;3(1):9-16³; and "Second Look at Utilization of a Closed-System Transfer Device (PhaSeal)", E. Thomas Carey et al., *Am J Pharm Benefits*. 2011;3(6):311-318⁴.

Basically in these studies^{3,4}, vials containing growth media were accessed 5 times during 7 days and each time a transfer of a small portion from the vial content took place using the Phaseal® CSTD under ISO 5 air conditions. Membrane disinfections and other standard aseptic techniques were used as well. The vials, simulating the drugs, were incubated after the 7 days of testing and examined to demonstrate the ability to prevent vial contamination during 7 days of use.

Aim:

The aim of the current study was to perform a significantly more extreme-use-conditions test than worse conditions expected in hospital pharmacy settings and worse than conditions of the above referenced previous studies^{3,4}, thus assessing the ability of the Equashield® CSTD to prevent the contamination of parenteral drug products during 7 days of use.

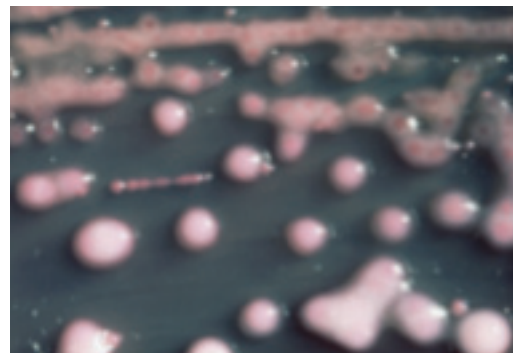
Method:

The challenge procedure consisted of inoculating the access membrane surface of multiple test article replicates of the Equashield® Vial Adaptor with gram positive or gram negative bacteria respectively, allowing the challenge to act and dry during ½ an hour, disinfecting the Vial Adaptor with a 70% isopropyl alcohol (IPA) pad, allowing the IPA to dry, connecting a new proprietary mating Equashield® 20ml Syringe Unit to the Vial Adaptor, and drawing a 4ml aliquot of sterile media (Soybean Casein Digest Broth) from the 50ml media-filled vial that the adapter was installed on. The test protocol specified a minimum inoculation titer of 10^3 colony forming units (CFU) per adaptor inoculation. The vials were divided into three similar groups and tested while the fourth group was added and tested in a replicated test one year later according FDA's request. The first vial access was on day 1 at the commencement of the 9 day test, when the Vial Adaptors were spiked onto the sterile media-filled vials and then incubated two days long. Following their incubation and starting on day 3 the Vial Adaptors were each subjected to a total of 10 or 7 or 5 (group respectively) inoculation-disinfection-access-transfer cycles over 7 calendar days, more specifically on days 3,4,5,8 and 9. The adapters were accessed once or twice each day, resulting in a total of 770 tested Equashield® Syringe Units with accesses and transfers.

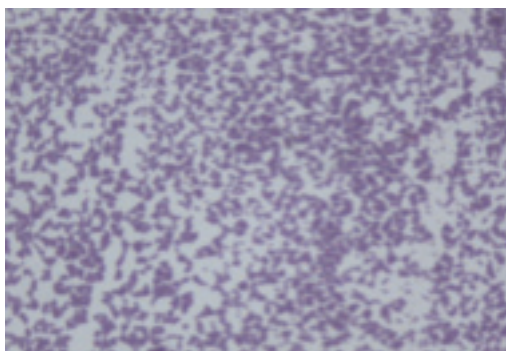
The first three groups were tested with *Staphylococcus aureus* (American type Culture Collection ATCC #6538) and group four was tested in addition to *S. aureus* with *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Pseudomonas Aeruginosa*.



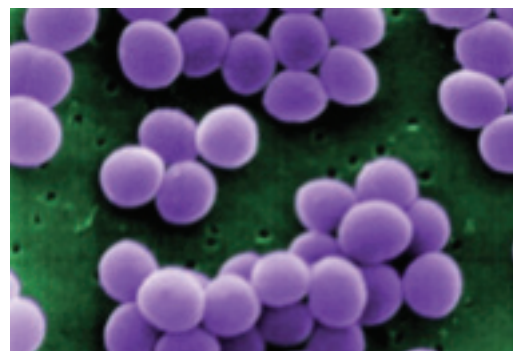
Pseudomonas Aeruginosa



Klebsiella pneumoniae



Staphylococcus aureus



Staphylococcus epidermidis

To maintain equivalence with clinical use in hospital pharmacy, ISO 5 air conditions were used in this study and only one person for each group performed the testing without assistance. Furthermore, approximately a third of the tested Equashield® syringes and adapters were old products with expired use-dates. The tested vials were incubated for 2 days at 30°-35°C after the 9 days of testing and examined to demonstrate the ability of the Equashield® CSTD to prevent vial contamination during 7 days of use.

Results:

All tested media-filled vials were negative for growth of any microorganisms.

Discussion:

The results of the study confirmed that despite the extreme-use clinical conditions, the Equashield® CSTD prevented the microbial contamination of media-filled vials, which are more sensitive to contamination than drugs. The penetration of even one microorganism is sufficient to create full contamination, and although the chances of bacteria survival and growth inside a hazardous drug vial are very slim, the media-filled vials used in the current study provided ample conditions for their multiplication and spreading.

To create even more extreme conditions, the vials in the current study were accessed up to 10 and 7 times during 7 days, whereas vials in previous studies^{3,4} were accessed only 5 times. Since the ability of a single connector to prevent contamination is naturally decreased with every additional connection and access, the number of connections gains increased significance in view of this non-linearly growing potential of contamination.

The repeated inoculation of the membrane with vivid bacteria before each of the vial accesses poses an extreme challenge to this primary microbial barrier; a challenge that does not exist (to such an extent) in a hospital routine setting, and that was also not included in the referenced studies^{3,4}.

Without the special mechanisms and design features incorporated into the Equashield® connectors, the membrane alone would not be capable of such barrier performance. In fact, the double-membrane connectors with their coring-free needles, special materials, and sequential guiding mechanisms, are responsible for creating the optimal conditions for satisfactory contamination prevention.

During the half hour that the bacteria were left to grow and settle on the membrane, the risk of them entering and settling in the openings or surface irregularities in the membrane, and subsequently moving into the vial or being moved by the penetrating needle, was obviously increased considerably in this study. In comparison, the FDA^{5,7} requires a minimum of only 1 minute for challenging microbial barriers. The ½ hour challenge time was chosen since at longer challenge the bacteria dries and becomes inactive.

The extreme conditions included the use of a significant number of Equashield® syringes and adapters that were old products with expired use-dates, whereby the sterility of their packaging may have been compromised, as often occurs with old products, and therefore the risk of contamination was much greater than expected from new products that were more recently packaged.

Unlike the conditions in many privileged hospital pharmacies and in previous studies^{3,4}, no cleanroom was used in this study and the laminar flow hood had an open front without the glass screen (which normally improves the isolation of the hood). To maintain equivalence with routine working procedures and conditions in a hospital pharmacy, only one person performed the testing without assistance, and the device packages were opened inside the hood. Furthermore, unlike experienced personnel in hospital pharmacies and previous studies^{3,4}, the operators were not proficient in using Equashield® and only received basic training at the commencement of the study.

The two types of vial accesses, namely: the spiking of the Vial Adaptor onto the vial and the access to the Vial Adaptor with the syringes, were purposely separated in this study. After spiking, the vials were incubated 2 days long and observed for turbidity before syringe accessing began. Both types of vial accesses were effective in preventing microbial ingress.

Furthermore, larger needles are known to cause greater damage and increase leak potential to barrier membranes than thinner needles; therefore Equashield® syringes with the larger needles were used in this study.

Thus, it could be argued that the current study not only reaffirms previous studies results, it also indicates that the solution in the vials remained uncontaminated when using the Equashield® CSTD in even more extreme conditions than may exist in hospital pharmacies, suggesting the possibility of practical benefit from the prevention of the microbial ingress feature, especially for single-use or non-preserved drugs.

Conclusions:

The current study unequivocally demonstrated that Equashield® CSTD is capable of preventing the microbial ingress and contamination of drugs over 7 days of use, which makes it a true CSTD, compliant with both aspects of NIOSH's definitions², namely: mechanically prohibiting the transfer of environmental contaminants into the system and the escape of hazardous drug or vapor concentrations outside the system.

Additionally, the standard disinfection procedure using IPA pads on Equashield® access ports was identified as adequate in preventing microbial ingress.

This ability of the CSTD, to provide extended microbial ingress prevention in extreme clinical conditions over a 7 day period, suggests improved patient protection in view of USP Chapter 7971 standards that currently limit the use of single-use non-preserved drugs to up to 6 hours in ISO class 5 air conditions.

Disclaimer:

The purpose of this research is to demonstrate the potential use of the Equashield Closed System Device. Its publication does not constitute and must not be construed as promotion or advice to deviate from, alter or otherwise exceed the guidelines, rules and standard related to aseptic preparations and techniques, or in any way deviate, alter or exceed the USE DATE or SHELF LIFE of the drugs. For any modification a user must comply with required steps and measures to change the established guidelines, rules and standard related to aseptic preparations and techniques, such as the procedural changes described in USP 797.

References:

1. United States Pharmacopeia. USP<797> Guidebook to Pharmaceutical Compounding – Sterile Product Preparations. Rockville, MD: United States Pharmacopeial Convention.
2. National Institute for Occupational Safety and Health Alert - Preventing Occupational Exposures to Antineoplastic and Other Hazardous Drugs in Healthcare Settings. DHHS (NIOSH) Publication No 2004-165; 2004.
3. McMichael D M, Moore Jefferson D, Carey E T, Forrey R A, Spivey S. M., Mulvaney J, Jorgenson J A, Haughs R D - Utility of the PhaSeal Closed System Drug Transfer Device. Am J Pharm Benefits. 2011;3(1):9-16.
4. Carey E T, Forrey RA, Haughs D, Moore Jefferson D, Jorgenson J A, McMichael D M, Mulvaney J M, Spivey S M - Second Look at Utilization of a Closed-System Transfer Device (PhaSeal). Am J Pharm Benefits. 2011;3(6):311-318.
5. Code of Federal Regulations. 1998. Subpart D - Microbiological Assay Methods, §436.103 - Test Organisms. 21 CFR Ch. I (4-1-98 Ed.).
6. United States Pharmacopeia 35 & National Formulary 30. 2012 - <797> Pharmaceutical Compounding-Sterile Preparations. United States Pharmacopeial Convention, Inc., Rockville, MD.
7. U.S. Department of Health and Human Services / Food and Drug Administration - Intravascular Administration Sets Premarket Notification Submissions [510(k)]. July, 2008.

Study reports:

1. Group 1 test report #680210.1 April 05, 2013; 10 connections and accesses (report attached).
2. Group 2 test report #680217.2 April 05, 2013; 7 connections and accesses (on file).
3. Group 3 test report #686405 May 07, 2013; 5 connections and accesses (on file).
4. Group 4 test report #13841-2 April 04, 2014; 5 connections and accesses (on file).

*Note: The testing of group 4 has been performed by Milouda & Migal Laboratories.



The tested vials were incubated for 2 days at 30°-35°C after the 9 days of testing and examined to demonstrate the ability of the Equashield® CSTD to prevent vial contamination during 9 days beyond the use date.

Results: All test device replicate media-filled vials were negative for any microorganism.

The negative controls were negative for growth. The positive controls were negative for growth. The bacteriostasis growth promotion controls which were media-filled vials inoculated with <100 CFU of the challenge organism and incubated for two days at 30-35°C were positive for growth.

Each of the test days, one device was inoculated with the test organism inoculum used that day and immediately placed into a bottle filled with 0.1% peptone water (PEPW). These were the positive monitor recovery controls used to establish the challenge titer. All were within the required parameters of at least 1×10^3 CFU per 0.02 mL inoculation throughout the test. For comparison, the challenge organism stock culture was serially diluted and titered directly.

All acceptance criteria were met:

1. The positive monitor recovery controls demonstrated at least 10^3 CFU per inoculated device.
2. The negative controls were negative for the challenge organism.
3. The positive controls were representative of the test set up. Due to the possibility of the organism getting trapped in the device, not all positive controls showed growth of the challenge organism. The test was valid, although not all positive controls showed growth.

Challenge Organism Inoculum Titers:

Organism	Identification	Day 3	Day 4	Day 5	Day 8	Day 9
<i>S. aureus</i> ATCC #6538	Positive Monitor Recovery Control- Calculated	9.2×10^3	8.6×10^3	1.1×10^4	8.7×10^3	6.3×10^3
	Direct Titer- Calculated	2.6×10^4	2.8×10^4	3.7×10^4	2.4×10^4	2.1×10^4

Note: Results are reported as CFU/0.02 mL device inoculation.

Microbial Barrier Performance Results:

S. aureus ATCC #6538:

Identification		Result
Test Article ID	Adapter Lot #	
Replicate 1	11-2013	No Growth
Replicate 2	11-2013	No Growth
Replicate 3	11-2013	No Growth
Replicate 4	11-2013	No Growth
Replicate 5	11-2013	No Growth
Replicate 6	11-2013	No Growth
Replicate 7	13-2995	No Growth
Replicate 8	13-2995	No Growth
Replicate 9	13-2995	No Growth
Replicate 10	13-2995	No Growth
Replicate 11	13-2995	No Growth



Laboratory Number 680210.1 Amended
 Microbial Ingress Test and Assessment for Extending the
 Beyond Use Date of Single-Use Vials Utilizing the
 Equashield® Closed System Drug Transfer Device Final Report

Identification		Result
Test Article ID	Adapter Lot #	
Replicate 12	13-2995	No Growth
Replicate 13	13-2995	No Growth
Replicate 14	13-2995	No Growth
Replicate 15	13-2995	No Growth
Replicate 16	13-2995	No Growth
Replicate 17	13-2995	No Growth
Replicate 18	13-2995	No Growth
Replicate 19	13-2995	No Growth
Replicate 20	13-2995	No Growth
Positive Control 1	13-2995	No Growth
Positive Control 2	13-2995	No Growth
Negative Control 1	11-2013	No Growth
Negative Control 2	11-2013	No Growth
Bacteriostasis Control 1	N/A	Growth
Bacteriostasis Control 2	N/A	Growth
Bacteriostasis Control 3	N/A	Growth

Amendment Justification: The minimum inoculation titer CFU was corrected in the summary section.