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EDITORS

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CONTENTS

Foreword
About the Council

1 On Pharma Island 12

EU Annex 1 Concerns and Comments
2 Annex 1 Barrier Technology Misinterpretation 16
3 An In-Depth Critique of Annex 1 2020 Consultation Draft 39
4 EU Annex 1 Concerns Regarding Particulate Air Quality and Environmental Monitoring Requirements 47
5 Comments on Annex 1 Sterile Medicinal Products 61

Regulatory Guidance, Sterilization and Aseptic Processing
6 "What, Not How" 73
7 Advancing the State of Aseptic Processing: Let's Get Serious 80
8 Redecorating the Decision Tree 86
9 . . . And the Award for Best Picture Goes To . . . 93
10 Cutting Things in Half 99
11 "Déjà Vu, All Over Again!" 106

Observations on Low Endotoxin Recovery and Regulatory Oversight

12 The Fallacy of Low Endotoxin Recovery 113
13 LER – Fact or Fallacy 118
14 LER Deconstructed 125

FOREWORD

The Council for Pharmaceutical Excellence (CPE) was formed to emphasize the importance of good science and engineering in the manufacture and analysis of pharmaceutical products. Our goals are to promote the use of sound, scientifically based technologies, engineering principles, manufacturing practices and effective quality assurance strategies for pharmaceutical products within a regulated environment. Central to these goals is the development of formal comments and position statements in response to regulatory guidance documents which impact those areas. The CPE believes that overly prescriptive regulations have handicapped the industry in numerous ways and thus have severely curtailed the use of sound science for further innovation and process improvement.

The Council seeks to accomplish this goal through:

- Publications on relevant subjects emphasizing the underlying science.
- Commentary on regulations, standards and guidance.
- Invited presentations at industry meetings.
- Transcripts/excerpts of interviews of CPE members.

- Participation on open meetings (in-person and virtual).
- Other appropriate means in furtherance of our goals.

This compilation of position papers, technical articles and internet posts represents the first Proceedings of the CPE. Herein, we present the views of Council members on subjects including manufacturing practices, Low Endotoxin Recovery, and commentary on draft EU Annex 1. Our stated opinions seek to improve the quality of pharmaceuticals and biopharmaceuticals while simultaneously reducing cost and improving patient safety.

The authors hope you find these ideas useful and urge you to submit your own, in the form of brief articles and commentary for potential inclusion in future Proceedings.

The Council operates virtually with no overhead and invites likeminded individuals to join us in bringing forth a new pharmaceutical age where science and engineering foster advancements in facilities, systems, equipment and methods that will provide products of higher quality and lower cost.

REM/JPA
5/21/2020

ABOUT THE COUNCIL

Dr. Jim Akers earned a Ph.D in medical microbiology with a research concentration in virology at the University of Kansas School of Medicine. After working in academics for several years at the East Carolina School of Medicine, Dr. Akers moved to the pharmaceutical/biopharmaceutical industry in 1981 where he held several positions in development, quality, validation, technical transfer, and regulatory operations. Dr. Akers served as President of PDA from 1991–1993 and served for many years on that organization's Board of Directors. He continues to serve on the Microbiology Expert Committee of the United States Pharmacopeia Expert Committee on Microbiology as he has since 1995, and also served as that Committee's Chairman from 2005 to 2015. Dr. Akers has authored over 100 publications and written more than 30 reference book chapters as well as editing two books. His principal areas of interest are aseptic processing, sterilization, contamination control, cell processing, and analytical microbiology. Dr. Akers owns and operates a consulting practice, Akers Kennedy and Assoc., LLC. He may be reached by email at *j.e.akers@icloud.com*

James Agalloco is President of Agalloco & Associates, which provides a range of technical services to the pharmaceutical and biotechnology industry. Since the formation of Agalloco & Associates in 1991, Jim has assisted more than 200 pharmaceutical, biotechnology, medical device, equipment manufacturers and bulk pharmaceutical firms with validation, sterilization, aseptic processing, compliance and related areas. Jim has more than 45 years of industry experience. He was formerly Director, Worldwide Validation and Automated Technology for Bristol-Myers Squibb. In that capacity, Jim was responsible for global process and computerized systems validation, as well as process automation efforts at several facilities. Prior to joining Squibb in 1980, he was employed in a variety of positions involving organic synthesis, pharmaceutical formulation, pharmaceutical production, project and process engineering at Merck and Pfizer.

He received a BE in Chemical Engineering from Pratt Institute in 1968 and his MS, also in Chemical Engineering from Polytechnic Institute of New York in 1979. He received his MBA in Pharmaceutical Studies from Fairleigh Dickinson University in 1983.

Jim is a past President of the Parenteral Drug Association and served as an Officer or Director from 1982 to 1993. He has served as a chairman and member of numerous PDA committees and remains active on several committees. He was made an Honorary Member of PDA in 2012. Jim is a member of USP's Microbiology Expert Committee (2005–2020). He serves on the Editorial Advisory Boards of Pharmaceutical Technology and Pharmaceutical Manufacturing.

Jim participates on the Scientific Advisory Boards of: MEDInstill, an innovative drug delivery company; and Eniware, a developer of novel sterilization equipment.

He has authored or co-authored more than 40 book chapters, over 140 papers and has lectured extensively on sterilization, aseptic processing and process validation at various PDA, PMA, and PharmTech meetings, domestically and internationally. He is co-editor of *Validation of Pharmaceutical Processes*, 3rd edition published in 2007 and co-editor of *Advanced Aseptic Processing Technology*, first published in 2010.

Phil DeSantis is a pharmaceutical consultant, specializing in Pharmaceutical Engineering and Compliance. Phil retired in 2011 as Senior Director, Pharmaceutical Engineering Compliance for Global Engineering Service at Merck (formerly Schering-Plough) located in Whitehouse Station, NJ. His responsibilities included development, implementation and support of standards and practices for all facility and equipment-related capital projects and site operations. He served as Global Subject Matter Expert for Facilities and Equipment and on the Global Validation Review Board and Quality Systems and Standards Committee.

Phil is a chemical engineer, having received a BSChE from the University of Pennsylvania and an MSChE from New Jersey Institute of Technology. He has over 50 years of pharmaceutical engineering industry experience. Prior to Schering-Plough, Phil held executive positions for Fluor Corporation and Raytheon Engineers & Constructors, where he led groups providing validation and compliance consulting services to pharmaceutical and biotech clients. Prior to that, he served in technical positions in several major pharmaceutical firms, including Squibb, Ortho Pharmaceutical Corporation and an earlier period at Merck. He is Chair of the PDA Science Advisory Board and is active in the International Society for Pharmaceutical Engineering (ISPE). He has been as a frequent lecturer for both organizations. He has published or contributed to several articles and books in the area of validation and pharmaceutical engineering. In addition, Phil has lectured on "Steam and Dry Heat Sterilization" as part of the FDA's field investigator training program.

Phil's background includes pioneering work in the genesis and evolution of process validation. His work in this area has led to broad expertise in all aspects of pharmaceutical manufacturing that support and ensure reliable and effective processes. Currently, Phil's primary focus areas of GMP Services include facilities and equipment, quality systems, project management, and professional development.

Russell E. Madsen is President of The Williamsburg Group, LLC, a consulting firm located in Gaithersburg, Maryland. Prior to forming The Williamsburg Group, he had served PDA as Acting President and was Senior VP Science and Technology. Before joining PDA, he was employed by Bristol-Myers Squibb Company as Director, Technical Services, providing technical and general consulting services to Bristol-Myers Squibb operations, worldwide. He is Vice-Chairman of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products, a member of the USP Microbiology Expert Committee, Chairman of the USP Visual Inspection of Parenterals Expert Panel, a member of Pharmaceutical Technology's Editorial Advisory Board, and an Honorary Member of PDA. He holds a Bachelor of Science degree from St. Lawrence University and a Master of Science degree from Rensselaer Polytechnic Institute.

Karen Zink McCullough is owner and principal consultant at MMI Associates located in Whitehouse Station, NJ. Ms. McCullough's consulting practice is specializes in Quality Systems building and remediation, and Pharmaceutical Microbiology, with a focus on the Bacterial Endotoxins Test.

Ms. McCullough has held a number of senior positions in the Pharmaceutical Industry including Vice President of Quality Operations at Dendreon, Sr. Director of Quality Compliance at Roche Molecular Systems and Director of Microbiology at Organon. She is a member of the USP Expert Committee on Microbiology, General Chapters and has served as a US representative to the revision of ISO 14698. She has published and presented extensively on the Bacterial Endotoxins Test. Ms. McCullough received her BA in Bacteriology from Rutgers University and her MS in Molecular Biology from the University of Oregon.

Robert Mello, Ph.D., is Principal Consultant at Mello Pharm Associates, LLC, serving the pharmaceutical and biopharmaceutical industry in the areas of microbiological drug product quality and regulatory affairs. Since 2015 he has been a member of the USP Expert

Committee, General Chapters – Microbiology. Prior to this he served 10 years at the FDA-CDER Office of Pharmaceutical Science's New Drug Microbiology Staff (now OPQ/Division of Microbiological Assessment) where he was a senior reviewer performing risk based quality assessments of the microbiology manufacturing and control information presented in INDs, NDAs, post-approval NDA supplements, and drug master files. Dr. Mello was also a major contributor to the 2012 FDA Guidance for Industry "Pyrogen and Endotoxins Testing – Questions and Answers." Prior to joining FDA, he was the Vice President of Education and Director of the PDA Training and Research Institute where he directed training programs for both industry and government health authorities. Dr. Mello has over 35 years of pharmaceutical industry experience in sterile product production, clinical supply, aseptic processing, sterilization, validation, facility design, QA/QC and regulatory affairs. Dr. Mello earned a Bachelor of Science degree in Biology from Providence College and a Ph.D. in Biochemistry from the Johns Hopkins University School of Medicine.

1

ON PHARMA ISLAND

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It is a difficult task to change the attitudes of regulators toward the science of microbiology. Many of the concepts expressed in Annex 1 and other regulatory guidance documents exemplify this. After submitting comments that never seem to result in needed change, we have often joked that we live in an imaginary scientific world which we have referred to as "Pharma Island." It's a world Lewis Carroll would find familiar.

On Pharma Island, microbiology is perfectly precise and sensitive. The limit of detection is zero and the standard error is <1 CFU. Thus, sterility is a measurable attribute, perfectly measurable. The mathematics of statistics doesn't apply on Pharma Island – the accuracy of counting actually increases as you move to smaller numbers of contaminants. Absence of contamination is sterility because we can measure to <1 CFU, leading to the erroneous conclusion that media fill contamination rates are the same thing as PNSU. "Absence of"

doesn't mean nothing grew, it means there are no microorganisms in the material period, full stop.

Furthermore, on Pharma Island the laws of thermodynamics can be bent to our will. Air is only unidirectional at 90 FPM. Linear regression is inferior to what we feel and the inverse of a number of organisms is directly convertible to a sterility assurance level. Movement of smoke in air is always a direct measure of sterility assurance, because on Pharma Island cold air does not sink; it follows air stream drawings. And warm air does not rise. On Pharma Island microbes are not only universally and easily measurable to one CFU, they can grow on anything: glass, plastic, stainless steel, or antibiotic powder, and they are so concentrated in air that they can penetrate HEPA filters with ease at any flow rate or pressure, even if the air is mostly recirculated and passes through the filters hundreds of times per hour.

And bacteria can spontaneously appear and grow without restraint anywhere they'd like without the need for nutrients or water. And every microorganism has extreme resistance to all disinfectants, and most sterilization processes as well. On Pharma Island any talk of water activity is a belief in witchcraft. Endotoxins can emerge fully formed and deadly on anything, anytime, anywhere. And if destroyed by chemical treatment they can magically reappear.

Good experimental design is whatever the regulators on Pharma Island deem it to be, and bad experimental design is also what they say it is. The same goes for processes and procedures. There's no need for testing or results: the outcome is preordained.

On Pharma Island there are extremophiles everywhere that can not only grow on anything but are unkillable and virulent at a concentration level of one cell. These deadly pathogens are untouchable by the human immune system and, although we don't know exactly what they are, their likely presence in any product at any time is an ever-present threat to public health. And if we should happen to detect even 1 CFU of almost anything we have to destroy the product and open a massive investigation to ensure it never appears again.

Actually, although our assays can find any microbial contamination reliably, there are, we must never forget, viable but

non-culturable organisms which are the exceptions which prove the rule, whatever the regulators say are the rules. This assures that there is never any level of testing that is sufficient and there is no optimum level of testing.

On Pharma Island injections can be given through the skin without introducing any contamination at all and all mammalian blood is sterile, always, at all times. All humans, even a 30-year-old supermodel getting a Botox injection, have compromised immune systems and every injection is potentially deadly. And every oral and topical product needs to be sterile, because ingesting or absorbing even a single CFU could kill us.

On Pharma Island only the opinions of the regulators matter and those who disagree are dangerous heretics.

Sarcastic? Maybe. But if you are fair and objective you know the previous paragraphs hit close to home. We don't need to change conventional understandings or the scientific definitions we've used in microbiology, biochemistry or in engineering, we just have to blow up Pharma Island and make its then-displaced inhabitants rejoin the real world.

EU ANNEX 1
CONCERNS AND COMMENTS

The following commentaries were prepared in response to the February 2020 draft Annex 1 – Manufacture of Sterile Products by the European Medicines Agency. We had commented extensively on the previous draft in 2018 and intended to do so again. We soon realized that the majority of the detailed comments we previously submitted were not adequately considered and shifted out approach. Our intent in these documents is to express our concerns comprehensively in a manner that line-by-line comments could never adequately accomplish.

2

ANNEX 1 BARRIER TECHNOLOGY MISINTERPRETATION

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INTRODUCTION

The treatment of emerging aseptic technologies in both draft Annex 1 revisions released since 2017 has poorly interpreted the meaningful differences not only between these technologies and manned cleanrooms, but also between the different technologies themselves (EC, 2017; 2020). The adverse impact of personnel, even when aseptically gowned, on the ability to prepare aseptic products safely has been recognized for several decades (Agalloco and Gordon, 1987). To reduce or ultimately eliminate the contamination risk from operators, there has been a steady progression in technologies that have endeavored to reduce the impact of aseptically-gowned personnel through physical means of separation.

Table 1 Aseptic processing technology comparison

	Figure 1	Figure 2	Figure 3	Figure 4	Figure 5	Figure 6	Figure 7	Figure 8	Figure 9
	Cleanroom	Cleanroom w/barriers	Passive RABS	Active RABS	Active RABS	Active RABS w/Room	Open Isolator	Closed Isolator	Closed Robotic Isolator
Aseptic Personnel	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Decontamination	Manual	Manual	Manual	Manual	Automated	Automated w/room	Automated	Automated	Automated
Material Transfers	Manual	Manual	Manual	Manual	Manual/RTP	RTP	RTP	RTP	RTP
Background	N/A	ISO 5	ISO 5	ISO 5	ISO 5	ISO 7	ISO 7-8	ISO 7-8	ISO 7-8
Differential Pressure	No	No	No	No	No	No	Yes	Yes	Yes
Open door activity	N/A	Yes	Yes	Yes	Rare	Rare	No	No	No
Air overspill	N/A	No	Yes	Yes	Yes	Yes	No	No	No
Line set-up	Aseptic	Aseptic	Aseptic	Aseptic	Non-aseptic	Non-aseptic	Non-aseptic	Non-aseptic	Non-aseptic
Leak Test	No	No	No	No	No	No	Yes	Yes	Yes
Glove Sterilization	N/A	N/A	Remote	Remote	Remote	Integrated	Integrated	Integrated	No Gloves
Fill Part Sterilization	Remote	Remote	Remote	Remote	Remote	Integrated	Integrated	Integrated	Integrated

Aseptic Personnel are personnel required to wear aseptic gowning
Decontamination the means for decontamination (manual or automated) and the target environment
Background classification of the environment where personnel are routinely present
Differential Pressure does a defined pressure differential exist between the background environment and the critical zone
Open Door Activity is an open door aseptic activity ordinarily required post decontamination
Air overspill does the system rely on air overspill to protect the critical environment
Line set up under what condition is the filling line configured for use
Leak test can the system be leak tested to confirm its integrity
Glove sterilization how are the system gloves sterilized
Fill part sterilization how are the fill parts sterilized

The important elements of aseptic processing environments have been outlined in Table 1, described more fully below, and visually depicted in both photographs and line drawings included in this narrative (see Figures 1–9). These images use a cartoon character shedding contamination. This is a visual reminder of the reality that aseptically gowned and qualified operators shed microorganisms and particles constantly (Whyte, 1999; Ljungqvist and Reinmüller, 2003). The first images (Figures 1–3) depict older designs that are in our opinion functionally obsolete. In the latter images (Figures 7–9), the operator need not be aseptically garbed, but the complete separation of personnel from the critical environment prevents the introduction of contamination. It must be recognized that no system that allows for operator access is truly protected from contamination ingress. The descriptions and images should be understood as generic examples and the details of any individual system vary and may include elements associated with a different example. The overall hierarchy of systems shown here is consistent with ISO 14644-7, Cleanrooms and Associated Controlled Environments – Part 7: Separative devices, which describes cleanroom technologies with respect to the type of separation, the means for confirming the separation and the effectiveness of the separation (ISO, 2004). Figure 10 adds overlays to Figure A.1 from the ISO14644-7 standard to highlight the significant performance distinction between RABS and isolators. Because RABS do not maintain a defined pressure differential between critical surfaces and personnel they cannot control human contamination as effectively as isolators. Only in their most evolved state do RABS designs approach isolator-like capability.

Manned cleanrooms which lack any meaningful separation between gowned personnel and critical surfaces are believed to perform less capably than even the least capable RABS design. Ordinary manned cleanrooms do not appear in Figure 10, as they are not considered separative environments.

CONVENTIONAL MANNED CLEANROOM

The very first cleanrooms provided little or no physical separation between personnel and the critical zone, relying almost entirely on unidirectional air, aseptically garbed personnel and good aseptic technique to protect sterile materials. All activities required must be performed by the operator. Most of these now incorporate partial barriers (flexible and/or rigid) to provide some minimal protection to the critical zone. These barriers serve primarily as a visual reminder to the operator because physical access is unrestricted.

Figure 1A Conventional manned cleanroom

Figure 1B Conventional manned cleanroom

Table 1 Excerpt	
Conventional Manned Cleanroom	
Aseptic Personnel	Yes
Decontamination	Manual
Material Transfers	Manual
Background	N/A
Differential Pressure	No
Open door activity	N/A
Air overspill	N/A
Line set up	Aseptic
Leak Test	No
Glove Sterilization	N/A
Fill Part Sterilization	Remote

MANNED CLEANROOM WITH BARRIERS

A common refinement to the manned cleanroom was the addition of rigid barriers separating the operator(s) from the critical zone. These barriers can be safety interlocked with the internal machinery to pause its operation while open. These barriers are opened to allow the operator unimpeded access for set-up, and the conduct of inherent and corrective interventions.

Figure 2A Manned cleanroom with barriers

Figure 2B Manned cleanroom with barriers

Table 1 Excerpt

Manned Cleanroom w/Barriers	
Aseptic Personnel	Yes
Decontamination	Manual
Material Transfers	Manual
Background	ISO 5
Differential P	No
Open door activity	Yes
Air overspill	No
Line set up	Aseptic
Leak Test	No
Glove Sterilization	N/A
Fill Part Sterilization	Remote

PASSIVE RABS

A separative design where sterilized gloves are affixed to the enclosure surrounding the critical zone. There is no separate air supply to the enclosure other than the HEPA-filtered air supplied to the room. These systems are typically manually decontaminated; line set-up and glove installation are performed aseptically. Depending upon the specific interventional activity doors may be opened. Component addition may or may not be performed without opening the enclosure.

Figure 3A Passive RABS

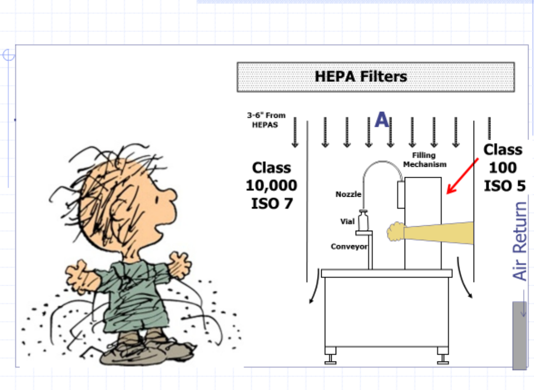


Figure 3B Passive RABS

Table 1 Excerpt	
Passive RABS	
Aseptic Personnel	Yes
Decontamination	Manual
Material Transfers	Manual
Background	ISO 5
Differential P	No
Open door activity	Yes
Air overspill	Yes
Line set up	Aseptic
Leak Test	No
Glove Sterilization	Remote
Fill Part Sterilization	Remote

ACTIVE RABS (MANUALLY DECONTAMINATED)

A separative design where sterilized gloves are affixed to the enclosure surrounding the critical zone. Air within the enclosure is HEPA filtered using air either drawn from room or separately from the air supply. These systems are manually decontaminated; line set-up and glove installation are performed aseptically. Depending upon the specific interventional activity doors may be opened though less frequently than with passive RABS. Component addition is more likely to be performed without opening the enclosure. When the doors are open, the operator is located within an external unidirectional air supply.

Figure 4A Active RABS

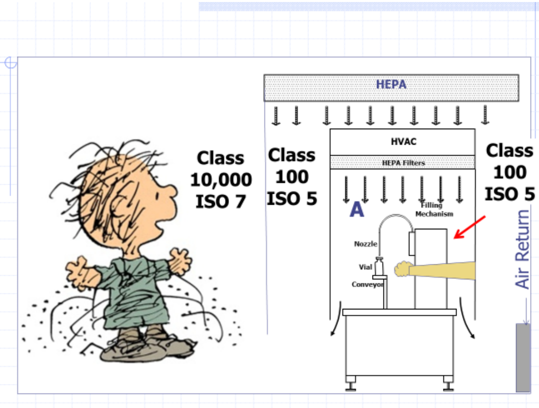


Figure 4B Active RABS

Table 1 Excerpt

Active RABS (manual decontamination)

Aseptic Personnel	Yes
Decontamination	Manual
Material Transfers	Manual
Background	ISO 5
Differential P	No
Open door activity	Yes
Air overspill	Yes
Line set up	Aseptic
Leak Test	No
Glove Sterilization	Remote
Fill Part Sterilization	Remote

ACTIVE RABS (AUTOMATICALLY DECONTAMINATED)

A separative design where gloves are affixed to the enclosure surrounding the critical zone. Air within the enclosure is HEPA filtered using air either drawn from room or separately from the air supply. Line set-up and glove installation are performed non-aseptically prior to decontamination. These systems are automatically decontaminated while closed, the system then relies air overspill to protect the critical zone during operation. Component addition is performed without opening the enclosure using isolator-like methods as the enclosure is not opened during operation. The described design is not in wide usage.

Figure 5A Active RABS (automatically decontaminated)

Figure 5B Active RABS (automatically decontaminated)

Table 1 Excerpt

Active RABS (automatically decontaminated)	
Aseptic Personnel	Yes
Decontamination	Automated
Material Transfers	Manual/RTP
Background	ISO 5
Differential P	No
Open door activity	Yes
Air overspill	Yes
Line set up	Aseptic
Leak Test	No
Glove Sterilization	Remote
Fill Part Sterilization	Remote

ACTIVE RABS (ROOM DECONTAMINATED)

A separative design where gloves are affixed to the enclosure surrounding the critical zone. Air within the enclosure is HEPA filtered using air either drawn from room or separately from the air supply. Line set-up and glove installation are performed non-aseptically prior to decontamination. The room and RABS are automatically decontaminated simultaneously, the system then relies upon air overspill to protect the critical zone during operation. Component addition is performed without opening the enclosure using isolator like methods as the enclosure is not opened during operation. The described design is not in wide usage.

Figure 6A Active RABS (room decontamination)

Figure 6B Active RABS (room decontamination)

Table 1 Excerpt

Active RABS w/room decontamination	
Aseptic Personnel	Yes
Decontamination	Automated w/room
Material Transfers	RTP
Background	ISO 7
Differential P	No
Open door activity	No
Air overspill	Yes
Line set up	Non aseptic
Leak Test	No
Glove Sterilization	Integrated
Fill Part Sterilization	Integrated

OPEN ISOLATOR

An isolated design where gloves are affixed to the enclosure surrounding the critical zone. Air within the isolator is HEPA filtered using air drawn from room. Line set-up and glove installation are performed non-aseptically and the isolator is automatically decontaminated. Component addition is performed without opening isolator using engineered systems for continuous ingress/egress. The isolator maintains a positive pressure during operation and is never opened during use. This design is in widespread use.

Figure 7A Open isolator

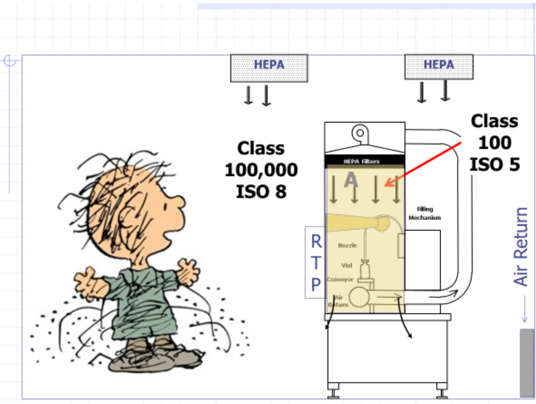


Figure 7B Open isolator

Table 1 Excerpt

Open Isolator	
Aseptic Personnel	No
Decontamination	Automated
Material Transfers	RTP
Background	ISO 7 8
Differential P	Yes
Open door activity	No
Air overspill	No
Line set up	Non aseptic
Leak Test	Yes
Glove Sterilization	Integrated
Fill Part Sterilization	Integrated

CLOSED ISOLATOR

An isolated design where gloves are affixed to the enclosure surrounding the critical zone. Air within the isolator is HEPA filtered using air drawn from room. Line set-up and glove installation are performed non-aseptically and the isolator is automatically decontaminated. Component addition is performed without opening the isolator using specifically designed batch transfer systems for ingress/egress. The isolator maintains a positive pressure at all times and is never opened during use. This design is in use globally for batch operations such as cell processing, API manufacturing, clinical or small-scale manufacturing as well for research.

Figure 8A Closed isolator

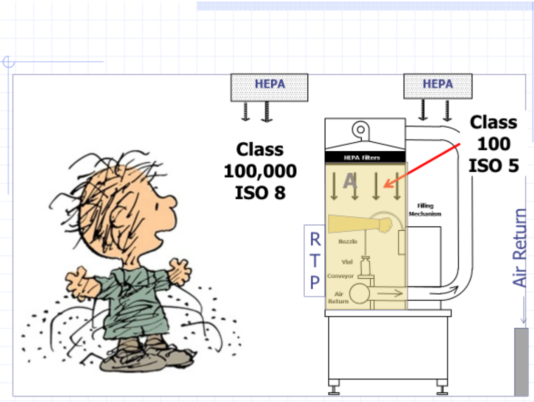


Figure 8B Closed isolator

Table 1 Excerpt

Closed Isolator	
Aseptic Personnel	No
Decontamination	Automated
Material Transfers	RTP
Background	ISO 7 8
Differential P	Yes
Open door activity	No
Air overspill	No
Line set up	Non aseptic
Leak Test	Yes
Glove Sterilization	Integrated
Fill Part Sterilization	Integrated

CLOSED ROBOTIC ISOLATOR

An isolated design where no gloves are required. All internal activities are managed by either robotic or machine automation. Air within the isolator is HEPA filtered. Line set-up is performed non-aseptically and the isolator is automatically decontaminated. Component addition is performed without opening the isolator using specifically designed batch transfer systems for ingress/egress. The isolator maintains a positive pressure at all times and is never opened during use. This design is in expanding usage especially for smaller batch sizes.

Figure 9A Closed robotic isolator

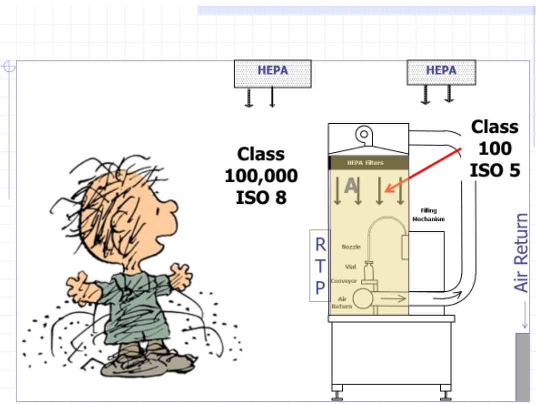


Figure 9B Closed robotic isolator

Table 1 Excerpt

Closed Robotic Isolator	
Aseptic Personnel	No
Decontamination	Automated
Material Transfers	RTP
Background	ISO 7 8
Differential P	Yes
Open door activity	No
Air overspill	No
Line set up	Non aseptic
Leak Test	Yes
Glove Sterilization	No Gloves
Fill Part Sterilization	Integrated

A CONTINUUM ISN'T CONSTANT

While several of these systems might appear very similar in appearance, there are substantial differences in how they operate that are critical to their performance and make a singular narrative inappropriate. Consider the following important features:

- *Is the assembly of the line accomplished aseptically*?
  Systems requiring aseptic assembly do not adequately separate personnel from critical materials and surfaces. They provide roughly the same performance as a manned cleanroom.

- *Is opening of the enclosure necessary during use*?
  Opening of the enclosure during processing compromises the barrier. These systems provide inadequate separation of gowned personnel from sterile materials.

- *Is the system manually decontaminated*?
  Reliance on gowned personnel to decontaminate the critical environment is less reliable than automated treatment. Locations can be missed, and re-contamination can occur.

- *Can the system be leak tested*?
  The adequacy of a separative device is affirmed by the ability to conduct a periodic leak test to confirm the integrity of the designed safeguards. Systems that cannot be leak tested increase contamination risk.

- *Is the system protected by air overspill*?
  Reliance on air flow to protect sterile materials surfaces is less certain than use of a controlled and alarmed pressure differential.

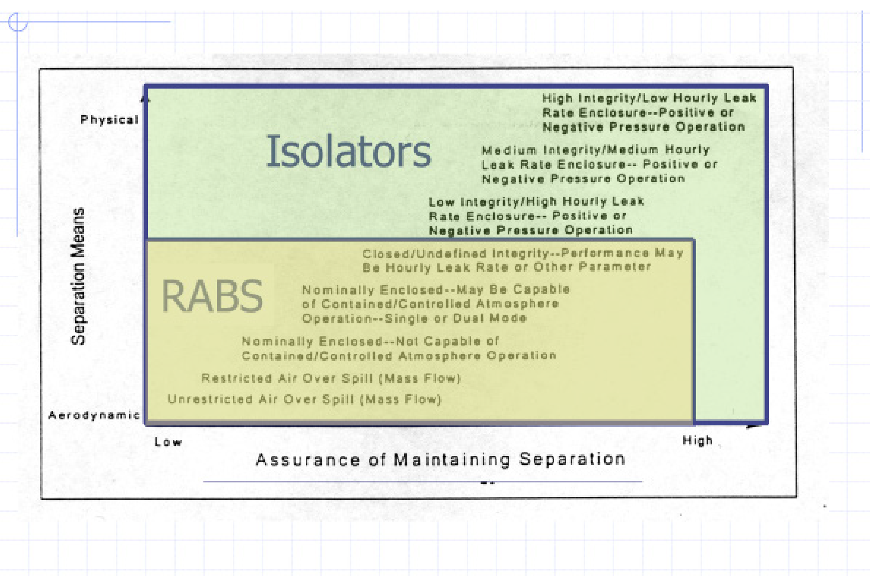
- *Are interventions with enclosure gloves required*?
  The elimination of gloves removes the most vulnerable component of many separative designs.

The answers to these questions reveal the true capabilities of the system (see Table 2).

Table 2 Key technical differences

	Cleanroom	Cleanroom w/barriers	Passive RABS	Active RABS	Active RABS w/RABS decon	Active RABS w/room decon	Open Isolator	Closed Isolator	Closed Robotic Isolator
Is the assembly of the line accomplished aseptically?	Yes	Yes	Yes	Yes	No	No	No	No	No
Is opening of the enclosure necessary during use?	Yes	Yes	Yes	Yes	No	No	No	No	No
Is the system manually decontaminated?	Yes	Yes	Yes	Yes	No	No	No	No	No
Can the system be leak tested?	No	No	No	No	No	No	Yes	Yes	Yes
Is the system protected by air overspill?	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No
Are interventions with enclosure gloves required?	N/A	N/A	Yes	Yes	Yes	Yes	Yes	Yes	N/A

Figure 10 Adapted from Figure A-1 from ISO 14664-7 – Separative Devices



The mere presence of a "barrier" between the operator and critical surfaces does not establish its adequacy for aseptic processing. Considering very different systems under a broad term "barrier technologies" when they differ substantially in their ability to produce sterile materials suggests an interchangeability that does not exist. The most capable of these systems represent "state-of-the-art" designs, while the least capable designs may be only marginally better than a manned cleanroom. What makes any separative technology better than a manned clean room is simply the elimination of direct interventions – full stop. Many of the cautionary statements in the Annex 1 draft relate to the least capable designs, which when applied to the most capable systems adds compliance driven complexity without any reduction in patient risk.

RABS are described as systems where personnel access to the critical environment is "restricted" by their configuration and operating practices, however they are only advanced aseptic technology systems if they completely eliminate interventions. Isolators provide for absolute separation between personnel and

sterile materials. The difference between "restricted access" and "no access" is infinite; therefore, any RABS design that allows access for direct intervention is more risk intensive than any isolator. It's ludicrous to even mention the lesser RABS configurations in the same context (Bozenhardt and Bozenhardt, 2019; Kline, 2010).

The Annex 1 draft substantially overstates the performance of RABS relying heavily on characteristics of the most capable systems to describe their operation. While we acknowledge that RABS with these operational characteristics are in operation, we believe these RABS designs represent only a small fraction of the installed base of RABS worldwide (Lysford, 2010). In our experience, there are substantially more RABS designs in current operation which not only allow direct operator intervention, but actually couldn't operate without them. The draft Annex 1 document fails to recognize that RABS encompasses a variety of design configurations and that the vast majority of RABS offer only minimal improvements over manned cleanrooms with partial or complete barriers. Further, the document does not distinguish between intervention dependent RABS and the acknowledged superior isolation technology systems. This means the authors are unable to discern or comprehend the real difference in risk mitigation between those systems which are truly advanced and those which are still direct intervention dependent and therefore heading toward obsolescence. These are egregious mistakes because they can encourage firms to adopt less capable systems with the belief that from the perspective of the EMA they are equal in performance (see Figure 10). The salient advantages of isolators over even the most capable RABS designs are the defined pressure differential between interior and exterior and the near total physical separation from the surrounding environment during operation. The best systems automate everything from set-up to lyophilizer loading and completely eliminate manual interventions. This is not a trivial difference; it is a major advance in performance.

UNIDIRECTIONAL AIR

The Annex 1 draft emphasizes the importance of unidirectional air in many locations. While we are in general agreement with this expectation in all applications where aseptically gowned personnel are present within the same environment as sterile surfaces, extending that requirement to all aseptic systems adds complexity, size and operating difficulties where that is not the case. The core premise behind the use of unidirectional air in cleanrooms is to minimize the potential re-entrainment of contamination and deposition onto sterile surfaces. The use of unidirectional air intends to direct air from its point of initial supply, past critical surfaces, past gowned personnel and then out of the critical environment. When personnel are wholly excluded from the critical environment by physical separation and differential pressure in an environment that is devoid of microorganisms, such as within an isolator, unidirectional air provides no improvement in performance (Agalloco, 2016). The addition of unidirectional air to an isolator, while seemingly simple, has several negative consequences including: increased system size; more difficult cleaning/changeover; increased operating cost; and slower decontamination/aeration. Systems where gowned personnel are never present, such as gloveless robotic isolators, are negatively impacted with no benefit whatsoever. Separate treatment of isolators and less capable barrier systems would allow the Annex 1 document to address this subject with less confusion.

MAJOR COMMENTS

While we fully intend to submit detailed comments to EMA, it is beneficial to include specific comments relative to these important technologies in this publication. Given the more general nature of these comments we have not identified specific sections or lines.

Separation of isolator and RABS content

The significant performance differences between isolators and the majority of lesser barrier system designs makes their combined

treatment in the draft less useful. This is particularly important as the document makes little distinction between the least capable (and unfortunately more commonly utilized) RABS and those described in the document. We would agree that highly capable RABS systems exist and that these designs perform at levels comparable to that of true isolators. We do not agree that these systems are representative of the significantly larger number of less capable RABS. The draft attempts to accomplish too much by embracing such a broad spectrum of design and performance. In doing so it fails to address the many important operational difficulties associated with most RABS designs which can affect patient safety. Weak RABS designs have limitations in decontamination access, glove installation, need for aseptic set-up and more frequent need for interventions with an open-door during processing. While highly capable RABS do exist, separate treatment of RABS and isolators is most appropriate. This would also ease the difficultly in allowing for non-unidirectional air with isolation technology.

System integrity testing

With the exception of RABS designs that are closed for decontamination, which are comparatively few in number, there are no means for integrity testing of RABS. This is an easily accommodated requirement with Isolation technology as they are decontaminated while closed, and leak testing can be integrated into every decontamination cycle.

Intervention contamination risks

The use of separative technologies such as RABS and isolators (aside from closed robotic isolators) does not directly reduce the need for interventions within. Separative technologies however can nearly eliminate the contamination risk associated with interventions by removing direct human interaction. Those technologies that eliminate operator access to the critical zone are more effective in reducing that risk. Reductions in the number of interventions can

only be accomplished by superior equipment design, automation and robotic manipulation and the use of components with fewer physical defects.

Invasive activities in system usage

The use of separative technologies must be looked at holistically, including all activities, not just those entailed in aseptic filling. The system must include other actions such as: line clearance; removal, cleaning and re-installation post-sterilization of product contact equipment; in-situ cleaning and decontamination of fixed equipment and enclosure surfaces; removal, cleaning, reinstallation and leak checking of gloves; set-up of the aseptic process including introduction and removal of set-up components and introduction, execution and recovery of environmental monitoring within the enclosure. Selection of an appropriate aseptic filling technology must consider how these activities are best accomplished. There should be a clear preference for those system designs that allow for these activities to be performed either before decontamination or in a manner that does not require access within the enclosure. That is typically only achievable in isolators and the limited number of RABS systems that are able to eliminate all operator access during use. The draft Annex 1 document largely ignores these activities, which can reveal the inadequacies in the design of many RABS designs.

Isolators are closed systems

The draft document presents two divergent opinions on whether isolators are considered "closed systems" or not. The introduction to the closed system content does not mention them, nor does the content within that section. In the definitions of closed systems, it specifically excludes isolators as a "barrier technology." The included definition of an isolator is ambiguous in this regard as it includes the following: "*Closed isolator systems exclude external contamination of the isolator's interior by accomplishing material transfer via aseptic connection to auxiliary equipment, rather than use of openings to the surrounding*

environment. Closed systems remain sealed throughout operations." This definition is consistent with wider industry usage which largely follows the perspective in PDA TR #28 revision where the important characteristics that establish a system as closed are provided (PDA, 2006). The industry considers isolators both batch and air sealed as "closed systems" (Agalloco, 2004; Agalloco et al., 2007).The essential factor is preventing the ingress of external contamination, and studies have confirmed that both air seal and closed batch isolators do this very well.

The isolator is "closed" to personnel which is the most important feature of any isolator. Experience has shown with isolation technology that airborne contamination from a surrounding environment is not a measurable source of contamination. Further, the closed system definition provided in the draft provides examples, but does not outline how those, or other systems could be established and verified as closed.

CONCLUSION

The Annex 1 draft document is deficient in its treatment of these essential technologies which are rapidly becoming CGMP for the preparation of sterile products. The final document can be expected to set a standard for sterile products facilities for decades to come. For it to be so poorly aligned with contemporary usage and practice for separative technologies is most unfortunate. The RABS models which underlie much of the content are not in widespread use, and recommendations based upon their unique operational features imply that the more prevalent and less capable RABS designs are their equal. This is a gross overstatement that bears addressing. The inclusion of isolators, in the confusion that is the content on barriers, is wrong as well. Isolators are universally acknowledged to be superior in performance to even the most capable RABS designs. In fact, the highest praise given to a RABS design is that it "provides isolator-like performance." ISO 14644-7 describes these systems as existing in a continuum, however for something as critical as the production of sterile drug products by aseptic processing, lines of demarcation must be drawn to assure that an appropriate design

is chosen. The Annex 1 draft fails badly in making that distinction. Most RABs systems are effectively clean rooms with doors that can be shut, and then reopened on demand. These are quite simply not advanced aseptic processing systems, but rather minor improvements on traditional clean rooms.

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3

AN IN-DEPTH CRITIQUE OF ANNEX 1 2020 CONSULTATION DRAFT

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INTRODUCTION

In late December 2017, the EMA issued a draft revision of its primary guidance on sterile products: Annex 1 – Sterile Medicinal Products (EC, 2017). The draft document was intended to update the existing Annex by providing more comprehensive and contemporary treatment of the subject matter. Comments were solicited from stakeholders in an effort to provide a finished document encompassing the experience range of all. This resulted in more than 6,200 comments from 140 different contributors and culminated in a revised draft issued in February 2020 (EC, 2020).

The authors, like many others, submitted formal comments to EMA on the 2017 version. In addition, we expressed our opinions publicly on that early version of the document (Madsen et al., 2018 a, b). With the 2020 draft now open for further comment, we believe it essential that the comments submitted to EMA be provided in a more detailed manner than EMA has requested. EMA desires this second consultation effort focus on 14 sections of the document and has specifically requested that comments made on the 2017 draft not be resubmitted. We believe this restriction is inappropriate because many of the comments we provided in 2018 were ignored. Therefore, not only will we again submit formal comments, we plan to follow a more aggressive path in providing constructive feedback on this latest draft.

Our planned approach will be as follows:

- Outline our overall concerns and general comments as they apply to the 2020 draft document (provided below).
- Issue detailed comments on the most troublesome portions of the draft with supportive rationale and comprehensive references for each.

We plan to post these comments on a biweekly basis.

GENERAL COMMENTS

- The 2020 draft has only a limited number of indirect references, leaving the readers with little opportunity to increase their understanding of the issues via the underlying referenced document(s). Reference usage was inconsistent in the 2017 version, but the removal of all references is a major setback. The 2020 revision should identify reference documents, and wherever possible peer reviewed, throughout that support the positions taken in the text.
- The document presents a view of sterile product manufacture inconsistent with that developed elsewhere, as codified in

regulations, international standards, and pharmaceutical compendia. For example:

- US and Japanese guidance on sterile product manufacturing differ markedly from what is presented (FDA, 2004; MHLW, 2011).
- The cleanroom content in the draft does not conform to the International Organization for Standardization (ISO) 14644 and perpetuates the myths that cleanrooms can be classified using microbiological results and that extensive microbiological testing in ISO 5 environments can enhance sterility assurance (ISO, 2015).
- United States Pharmacopeia (USP) chapters <1211>, <1228>, and <1229> provide more contemporary and scientifically sound guidance for sterile product sterilization, depyrogenation and preparation (USP, 2019; 2016; 2013).

• The 2020 draft of Annex 1 does not adequately address the substantial differences in aseptic processing technology currently available. Specifically, restricted access barrier systems (RABS) are described in a manner that suggests these systems are rarely opened and provide performance comparable to that of isolators. RABS when well-designed can approach isolator capabilities, however there are more numerous less capable open RABS designs in use that are minimally superior, if at all, to manned cleanrooms with barriers (Kline, 2010; Bozenhardt and Bozenhardt, 2019). The RABS systems described in the draft represent only a small fraction of the installed base as a simple internet search of RABS images will quickly demonstrate. RABS systems must be understood as more susceptible to human contamination given the "open-door" aseptic activities often needed for line clearance, glove exchange, product changeover, cleaning and decontamination. Isolators provide not only superior physical separation but a measurable pressure differential that makes them vastly to superior to the majority

of RABS designs (ISO, 2004). In addition, virtually all isolator systems employ automated decontamination system, something found only in a limited number of RABS (Lysford, 2010).

ISO 14644-7 makes it clear that there is a range of systems which can be classified as ISO 5, yet it is also evident that there are substantial differences in the level of assurance provided by them. Isolators represent the current pinnacle of performance for aseptic processing and that should be made apparent in the Annex. A similar perspective on isolation technologies' superiority can be found in PDA TR #34, which provides a more contemporary perspective on isolators although published 19 years ago (PDA, 2001).

Given the importance of physical separation in aseptic processing, the significant differences in performance across these systems should be acknowledged. That is not apparent as this document considers "barrier technologies" overall without discriminating amongst them.

- EMA's illogical and arbitrary insistence upon maintaining its Grade A/B/C/D system should be abandoned and the ISO 14644 classification system used throughout the document. Grades A, B, C, and D should be replaced with the corresponding classes of ISO 5, 6, 7, and 8. All references to the arbitrary and confusing Grade B, and the totally undefined Grade A/B, should be removed. The re-insertion of 5-micron particles into the draft for classification of environments resurrects an "urban myth" regarding their utility that offers no benefit to the user or the patient. The multiple revisions that Annex1 has gone through since its inception to impose a 5-micron target in classification is indicative of trying to maintain a poorly conceived and artificial concept despite its numerous limitations in analytical measurement, statistical reliability and utility. The poor recovery and low incidence of 5-micron particles are such that they are unreliable as a performance standard.

- Classification is a well-defined activity in the ISO 14644 series of standards. It is most reliably performed on systems in the "at rest" condition where the number of particles of a single size are used to establish the classification of an environment. The "at rest" state is easily reproduced as it is devoid of personnel who are the largest contributors of contaminants. As such it provides a reproducible baseline to establish the adequacy of the system's design and reassess it over time. Its greatest utility is in initial and periodic confirmation of the system's design and performance and is largely independent of operating practices. The "in operation" classification expectations in the draft require operator presence which introduces variables that can perturb the results without offering meaningful benefit. Monitoring entails assessment under operational conditions which are variable and incorporate factors related to the system owners operating practices (Agalloco et al., 2019). The draft Annex also uses identical limits for all classes in both the "at rest" and "in operation" states for classification (Table 1) and monitoring (Table 6). Table 1 (classification) should include only values for the "at rest" state, while Table 6 (monitoring) should only include the "in operation" state values.

- The document has a perspective on microbial monitoring that is inconsistent with scientific reality. The limit of detection for microbial testing is substantially higher than one colony forming unit (CFU), a level which permeated the 2017 draft. The 2020 version expects "no growth" on all samples for all ISO 5 microbial samples. This erroneous belief leads to numerous misconceptions and overstatements regarding what value environmental monitoring has in the preparation of sterile medicinal products. Aseptic processing systems have never been demonstrably absent of contamination, nor do they need to be, and thus this expectation is wholly inappropriate (Winterberg, 1989; Madsen and Agalloco, 2019; Hussong and Madsen, 2004). It is wrong to suggest that any environment in which humans work could be absent of recovered microorganisms even when a low resolution method such as EM is utilized.

- The most recent draft document has relocated content and is somewhat better organized than the initial version. Nevertheless, further improvement is needed especially as it relates to subsections within the larger categories as their hierarchy is not immediately apparent.

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4

EU ANNEX 1 CONCERNS REGARDING PARTICULATE AIR QUALITY AND ENVIRONMENTAL MONITORING REQUIREMENTS

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HISTORY AND BACKGROUND

EU Annex 1 "Manufacture of Sterile Medicinal Products" has been in operation since 30 May 2003. It underwent revision between 2005 and 2007 and after public review and comment was official as of 1 March 2009 with a provision for a one-year allowance for implementation on the new provisions for vial capping. The capping of vials in "Grade A" conditions proved controversial and was subsequently revised to allow capping under unidirectional HEPA-filtered air. The European Commission announced on 20 December

2017 the publication of the next revision of this important and widely followed global GMP document. Having received and considered public comments, EMA released a further revision which became available for review in February 2020 (EC, 2017; 2020).

In the preamble to the release of the 2017 revision of Annex 1 it was noted that the history of this guideline traces back to 1971. Unmentioned in that preamble is the fact that the document published in 1971 and from which the EU GMPs sprung was the UK "Orange Guide" which was British Good Manufacturing Practice (MHRA, 2017). The Orange Guide still exists and may, with the departure of the UK from the EU, return to its former primacy in the UK. It remains to be seen how the UK and EU will move forward with respect to pharmaceutical regulation.

This history of Annex 1 is important only in the sense that the areas of the document covered in this commentary, which are those associated with clean room operations and monitoring, have changed remarkably little over the decades. The Clean Room "A, B, C and D" Grades had their genesis in the UK Orange Guide, as did the longstanding emphasis on 5 µm particulates.

There has been discussion over the years about how and why the Orange Guide/Annex 1 requirements co-evolved without ever really being "harmonized" (MHRA, 2017). We should first discuss how much disharmony there really is between the Annex 1, FDA guidelines (FDA, 2004) and ISO 14644-1,2 (ISO 1999; 2015). The answer might surprise you. The Orange Guide predates ISO but not Federal Standard (FS) 209A-E (ISO, 2015). If the original version of EU GMPs was the Orange Guide, it can equally be said that the original version of ISO 14644 was just FS 209E (ISO, 1999) having undergone metric conversion. Class 100 from FS 209E called for a particulate air quality of 100 or less 0.5 µm particles/ft^3. This ratio was unchanged; it was simply corrected for a volume of M^3 in ISO 14644. Since Class 100 would no longer work after the metric conversion, the 1-9 ISO 14644 classes were borrowed from the Japanese clean room standard. Class 100 became Class 5 and Grade A, which had been equivalent to Class 100, became equivalent to the near identical Class 5.

The Orange Guide and FS 209 had diverged many years before ISO 14644 came into being. FS 209B was the last of the FS 209 series to include microbiological requirements. When FS 209C came along in 1987 the engineers and scientists reasoned that clean room designers couldn't be held responsible for designing a clean room to meet microbiological requirements. Therefore, they eliminated the microbiological control requirement from FS 209C, and those requirements never reappeared. When FS 209E (1992), the last of the series, was withdrawn in favor of ISO 14644 it had no microbiological requirements. Interestingly to this day neither does ISO 14644.

You might ask why those in charge of FS 209C took the step away from microbial classification requirements for clean rooms. We would argue because they were smart. They understood that microbial classification of clean rooms didn't make sense. Clean rooms were used for various purposes: electronics, aeronautics, space exploration, research and drug manufacturing. They reasoned that microbial requirements, if there should be any, should be industry specific. They also reasoned that as humans produced essentially all microbial contamination and engineers couldn't design to a microbiological specification without having control over gowning requirements and getting into process conditions. They'd have to know how many workers would be in a room and they already knew the presence of humans could cause issues with total particulate count. This is why clean rooms are tested at rest as well as in operation. We couldn't agree more with the team that produced FS 209C – they were right then. and they remain right today. They realized that there was only a correlation between microbial levels and particulate levels if humans were the primary source of both. If only the Orange Guide and the authors of Annex 1 had followed their lead.

FDA did follow their lead after a fashion. That agency in their guidelines loosely connected environmental monitoring (EM) expectations to microbial requirements but did not endorse microbial clean room classification. They certainly did endorse EM but not in the way the Orange Guide/Annex 1 did. From that moment on the Microbial Classification of Clean Rooms was essentially an Orange Guide/Annex 1 thing. They muddled classification and monitoring,

but even with that in mind EU, Japanese, and USA aseptic processing clean rooms operate in all essential ways the same. Those who audit those rooms don't see a real gap between actual operational conditions or performance. We see some cultural differences in pre-gowning, gowning types, use of air showers, and other minor details but basically, they all work the same way. Most importantly they function to the same level of capability. Why shouldn't they since all are designed to meet an ISO 14644-1,2 requirement for particle air quality including those that are labeled "Grade A" rather than ISO Class 5? In fact, a room labeled FS 209E Class 100 is able to meet the same minimum particulate air performance levels as well. There is no practical space among any of these standards in terms of total particulate air quality expectations.

BUT HOW ABOUT LARGER PARTICLES?

We'll not pull our punches; we are pleased to see the 5 µm particulate air quality testing requirement gone for ISO 5 (Grade A) and Grade B at rest. The use of 5 µm for classification should have been gone from Annex 1 in the previous revision and arguably should never have appeared in ISO 14644-1,2:1999. FS 209E, the final iteration of that influential series of standards, did not stipulate 5 µm particulate as a size class for the Class 100 clean room, which is equivalent in all key respects to ISO 5. However, in spite of the statistical and analytical equipment limitations associated with the analysis of 5 µm particles, EU Annex 1 and the Orange Guide before it maintained a zero 5 µm particulate limit. This was obviously a bad idea as the vendors of equipment noted electronic noise could give a spurious 5 µm signal, in other words a false positive. It was also noted that many particulate air quality measuring systems were unable to produce reliable results at 5 µm. We've never heard a scientifically valid explanation for the stubborn adherence to 5 µm sampling, this includes the stated belief that most bacterial contamination would be adherent to skin cells or would appear as clusters of cells. No studies of which we are aware have confirmed that supposition and in fact there are other studies that indicate the preponderance of airborne bacteria are probably planktonic (Ljundqvist and Reinmüller, 2003).

This aside, any individual scientist or engineer who has looked at as much clean room particulate sampling data as we have, knows that getting a 5 µm count has always been rare. Rare means statistically unreliable, which is why the ISO working group responsible for the ISO 14644 series 2015 revision dropped 5 µm. The ISO Class 5 classification target for 5 µm particles was 29, which has almost never been observed. The counting statistics are extremely unreliable with at such very low numbers of particles per M^3, thus in ISO 5 environments the standard deviation associated with 5 µm counting could approach 100%. Therefore, for ISO 5 rooms one should classify at smaller particulate size ranges where the counts are far more numerous.

EU Annex 1 retains a 5 µm requirement for Grade B in operation as well as Grades C and D, although this is consistent with the ISO 14644 2015 revision which has an established classification limit for 5 µm particles of 293 for ISO 6, and 2930 for ISO 7. There is no requirement in ISO 14644 to classify a room using multiple particle size ranges and it continues to make sense to test at size ranges closer to the maximum penetrating particle size of 0.3 µm.

EU Annex 1 hasn't left EMA's fixation on larger particles completely behind, however. They now suggest that consideration should be given using a larger particle size range for classification; they suggest one µm. This is pointless because that size is also well above the maximum penetrating particle size of 0.3 µm for HEPA filters. Testing at one µm or 5 µm are truly valueless endeavors.

We have a better idea. Annex 1 should simply cease and desist in its unique classification requirements. Those requirements are not significantly different from the ISO 14644 2015 revision. Table 1 should be deleted, as should all the discussion around classification. ISO 14644 provides perfectly valid information and guidance. The healthcare industry does not need two parallel ways to do the same thing. While they are at it, EMA should join the 21st century and put the Annex 1 Grade A, B, C and D classifications out to pasture. Grade A is really just renamed ISO 5. Grade B is a strange hybrid which approximates in most respects ISO 6, Grade C is ISO 7 and Grade D is ISO 8 at rest. There are no ISO 8 rooms in any aseptic processing area and Annex 1 is aimed at sterile product manufacturing. Grade D or ISO 8 may

be used in low criticality rooms in support of aseptic processing but outside the aseptic processing area, or perhaps for washing or cleaning of equipment in both aseptic and terminal sterilization operations. It could even be used as an Isolator background environment. However, these are low risk activities and there is nothing unique about Grade D compared to ISO 8. A further advantage of removing particulate air quality classification and the scientifically invalid notion of microbial classification would be that the recommendations in Annex 1 would be limited to monitoring (Agalloco et al., 2019).

AIR VELOCITIES

Air velocity has always been a curious inclusion in guidance documents or standards. Its origins are murky, but they seem to trace back to a recommendation coming out of Los Alamos national laboratories, where the HEPA filter was developed. A general finding was reported that 90ft/minute was a good compromise among noise, vibration, energy consumption, and effective removal of particulates. In other words, there is no magical correlation between air velocity and contamination control. All Annex 1's authors have done is again go back to an early version of FS 209B from 1972 and convert that standard's recommendations of 90 ft/min +/- 20% to metric velocities. 0.36–54 M/s converts to 90 ft/min +/- 20%. Perhaps our friends in Europe didn't notice that in the last two iterations of FS 209 this requirement was removed as it was determined it was not necessary for the government of the USA to enforce as a purchasing requirement. The FDA seems to have missed that message as well and lost sight of the fact that the original recommendation had nothing to do with aseptic processing.

A further problem here is imposing the velocity requirement at the working height. This requires increasing the velocity at the filter face to speeds far greater than those recommended by the engineers who wrote FS 209B, because at the work surface the air generally runs into processing equipment including conveyors, fillers, stoppering systems, etc. oriented at 90° to the direction of airflow. Therefore, air at any velocity directly meets blockages, resulting in sideways diversion of the air flow in the direction of the lower pressure air

returns. Accompanying this are unpredictable and unavoidable eddy currents formed by the air's encounter with process piping, vibrating equipment, moving conveyors and of course boundaries created by barriers of any kind, or isolator walls.

The higher velocities at the HEPA terminals required to achieve 90 ft/min at the work level increase fan power requirements, burn more energy, increase the facility's carbon footprint, increase vibration, add sensible heat to the factory and may even shorten the life of filter media and gaskets. Equally unreasonable is the imposition of a fixed requirement such as this for advanced aseptic processing systems that simply don't need this level of air movement due to the absence of working personnel. Annex 1 here is imposing a rigid requirement that was made flexible in FS 209 nearly four decades ago. Again, it's time to move into the 21st century or even the end of the 20th.

EM REQUIREMENTS

We are concerned about the establishment of uniform EM limits given the lack of uniform methods for conducting EM and the high variability, which is characteristic of plate counting, particularly where low numbers are recovered. We believe that regulatory requirements and industry practices have lost sight of the fact that microbiology has always been a logarithmic science. It is clear to us that the authors of Annex 1 profoundly lack that understanding. There are, as USP <1116> (USP, 2019) has manifested, more effective ways to assess facility hygiene, preferably ones that lacked the inherent arbitrariness of requirements and ignored analytical variability as well as the obvious statistical limitations.

From a clean room engineering and operations perspective, it has long been recognized that clean rooms of a given ISO or FS 209 classification level were not always equivalent. Given the relationship between the EU Annex 1 Grades and ISO and FS 209 classification requirements and metrics, it must be recognized, for example, that not all Grade A, B, C or D rooms are equivalent in terms of microbiological contamination management. Differences in gowning requirements, HEPA filter coverage, air exchange rate, personnel load and varying intended uses, e.g., aseptic compared

to bioburden control, mean that an ISO 7 environment in an aseptic processing area would perform very differently from an ISO 7 environment in a bioburden-controlled area. This applies equally to a Grade C environment which is functionally the same as ISO 7. In our opinion, this further supports the absurdity that is microbiological room classification.

The problems described in the previous paragraphs can readily be seen in the 2020 draft EU Annex 1 clean room "grades" where a considerable increase in microbial limits are evident between Grade B and Grade C. It is logical that the much larger increase in recommended limit between Grade B and Grade C as compared to Grade A and Grade B reflect an attempt to serve rooms of various classifications and usage under a single microbiological classification scheme. By logical extension this approach underscores the problem of attempting to correlate microbiological performance of a clean room with ISO classification rather than considering the laboratory or manufacturing purpose to which a room is assigned.

Realistically, the values for Grades B and C are unreasonable for aseptic processing areas. In Grade B rooms the vast majority microbiological samples will be zero. One would rarely expect to see a recovery of 50 or 100 CFU in an ISO 7 aseptic processing environment, but one may well see those levels reached in a bioburden control facility with less restrictive gowning requirements than those applied to aseptic processing. An even more significant source of wonderment is the Grade A (really ISO 5) limit of no growth. First, we must report that no growth in microbiology is not the equivalent of sterile. Second, the limit of detection of microbial assays used in EM is in the range of 10–100 CFU, which is why media fertility tests are generally expected to fall into that range. USP <1116> has suggested for years that 99% of the plates exposed in a clean room should be no growth, a standard that is regularly met in aseptic processing ISO 5 environments. However, the USP <1116> requirement allows for the possibility of a false positive while recognizing that in ISO 5 environments we are working at the limit of detection of these EM methods. It would be pointless for firms to need to write some kind of investigational report if they find a rare plate with a colony(s). A useful investigation at the ragged edge of measurement is pointless

because honestly it is impossible to really do so. This is an invitation not to processing quality but rather to busy work. In summary, the EM "limits" proposed in Annex 1 are useless, odd and scientifically invalid. Obviously, in ISO 5 aseptic environments where the USP <1116> requirement of <1% of the samples have growth is met, trend analysis is automatically accomplished because such low recovery levels make any real trend impossible. A no growth requirement accomplishes nothing except the removal of an allowance for an anomalous false positive and the appearance of, but not the reality of, greater rigor.

THE STATISTICAL PROBLEM

An oft cited article covering among other things the statistics of EM described and discussed the inherent statistical problem associated with attempting to draw strong quality conclusions from extremely low recovered counts (Hussong and Madsen, 2004). As noted previously microbiology is a logarithmic science, and we learn in our first university microbiology course that in a practical sense the best quantal range for serial dilution in plate counts is to arrive at counts on a spread plate in the range of roughly 25–30 CFU up to 250–300 CFU . Serial dilutions are often 10-fold and the reader will note that this quantal range covers a 10-fold or "one-log" range. The reason for this range is not just ease in counting, there is much more to it than that.

As Hussong and Madsen illustrate, at the extremely low colony counts seen on typical EM plate recoveries in aseptic processing, the inherent statistical variation is enormous. Table 1 below illustrates the expected variability at counts below the quantal range typically recommended for plate counting.

As stated above >99% of the plates in ISO 5 environments will have no colonies (this applies to the functionally identical Grade A as well). In the rare instances that colonies are present they are most often at 5 CFU or less. As demonstrated in Table 1 at a recovery of 5 CFU the standard deviation as a percentage of counts is 45%. This means that if a test recovered 5 CFU the one standard deviation error bars would extend from roughly 3 CFU to 7 CFU. At 3 CFU

an error bar would extend from 1–5 CFU and at 1 CFU the standard deviation would extend to 2 CFU.

Table 1 The standard deviation of average plate counts

Mean Count	% Standard Deviation	Actual Population
1	100	0–2
3	58	1–5
5	45	3–7
10	32	1–20

Adapted from Hussong and Madsen (2004)

Annex 1's authors have duplicated the mistake made in the aseptic processing guidance cGMP document published by the FDA in 2004. In both the Annex 1 draft and the 2004 FDA guideline ISO 5 target level (for Annex 1 limit) is zero CFU, which is a value the typical ISO 5 aseptic area provides more than 99% of the time. The FDA action level is stated as 1 CFU, the statistical issues associated with considering a 1 CFU result to mandate action should be obvious to any scientist. Annex 1 doesn't even contemplate a value greater than no growth, which is both analytically and statistically wrong.

THE SAMPLING PROBLEM

EM samples only a tiny amount of the air flowing through a clean room and generally much less than 5% even if samples are taken continuously. These samples reflect only a snapshot in time and cannot be reproduced. Taking another sample at the same general location days or weeks later is a pointless investigational tool because the same conditions that existed at the time of the original sample no longer pertain. This is equally true of surfaces which are regularly cleaned and/or disinfected. The "investigation" of EM results through resampling is scientifically pointless because to have

a correlation we must have a mutual relationship to the original sample which we can't have.

A modern ISO 5 Critical Zone in aseptic processing operating at about 0.4 M/s near the HEPA filter (12" or about 20 cm) will produce around 500 ac/hour. If we consider a 2.5 meter ceiling height and a 5 m^2 floor space this area would have a total volume of 12.5 m^3. This total critical zone volume multiplied by the 500ac/hour means that an air volume of 6250m^3 would pass through this space each hour. Therefore, if 10 continuous air samplers capable of sampling 5m^3 of air per hour were used it would be possible to test sample 50m^3 per hour. This would amount to a sample of 0.8% of the air passing through the room, which would hardly provide a means of assuring sterility. This illustrates the futility of attempting to use EM as an indicator of anything other than a crude assessment of facility hygiene, there is no amount of monitoring that could provide anything remotely like sterility assurance.

This is example also points out the absurdity of "resampling". If an aseptic processing area air sample was incubated for five days found to have a count and the same spot was resampled fully 60,000 volumes of fresh HEPA filtered air would have passed through this space between samples! It should be obvious to any scientist that there is no rationale for correlating these two very independent samples.

Also, this example points out the uselessness of passive air sampling, which remains a feature of Annex 1 after all these years. It's hard to imagine that passive sampling does anything but increase product risk in the critical zone requiring as it does interventions by personnel at multiple locations every 3–4 hours. These interventions for sampling are in many modern systems more numerous than actual aseptic processing work interventions. What could possibly be the scientific justification for this? This seems very much like a stubborn adherence to a practice which long ago outlived its usefulness.

THOUGHTS ON ADVANCED ASEPTIC PROCESSING

We have long been concerned that regulatory agencies and standard setting organizations have failed to recognize the clear scientific and engineering distinctions between advanced aseptic processing

systems and manned clean rooms. Annex 1's authors in the latest revision have again failed the industry and our patients by failing to draw a clear distinction between advanced aseptic processing systems which obviate the need for human occupancy of the clean space while at the same time eliminating direct interventions and less capable aseptic processing approaches which allow part- or full-time human occupancy. The need for full- or part-time human occupancy is typically accompanied by direct human intervention. We have recently covered these issues in great detail.

Our purpose here is to point out that the distinction between advanced and traditional aseptic processing must extend to microbial monitoring as well. We agree that all advanced systems should meet ISO 5 particulate air quality requirements. However, there should be no requirements for air velocity and openness to turbulent air flow for some applications. Most importantly though, EM should be greatly reduced or even eliminated in advanced aseptic processing systems. We predominately see null data (no growth) in the ISO areas of conventional, traditional human occupied aseptic processing facilities. At 99% no growth outcome, the need to sample is obviously lessened. In advanced aseptic processing systems, the need for microbiological monitoring should be reduced or even eliminated once the capability of the system is established. There are other metrics such as positive air pressure controls, and total particle counts to confirm performance of ventilation systems, that provide quality operational data without the handling of media. The reduction or elimination of EM sampling will reduce the cost of goods produced, which benefits patients, and it will also reduce energy consumption and waste which benefits our environment.

CONCLUSION

It is past time to bring our standards for aseptic processing into the 21st century and eliminate parallel "me too" standards which exist only to underscore political authority rather than promulgate good science, engineering and operational practice. Standards like Annex 1's revision stifle innovation, lock us into antiquated thinking, emphasize things that are not important and waste human, financial

and environmental resources. They do no good for our patients and when misapplied in inspections lead directly to unfortunate regulatory comments and in the worst of situations unnecessary enforcement action and even drug shortages. We can and should expect better.

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COMMENTS ON ANNEX 1 STERILE MEDICINAL PRODUCTS

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The authors have reviewed the February 2020 draft of EMA Annex 1 Sterile Medicinal Products in detail. We find the document to be severely lacking on many technical issues. Moreover, the document fails to consider the many improvements in aseptic processing which have occurred over the last 20 years. In this regard, it seems more plausibly written in 1985, rather than 2020. When we first received it, we fully intended to conduct a thorough and complete review as we did for the 2017 initial draft. Shortly after commencing that effort, we realized that the document was largely unchanged: in fact, the majority of the comments we submitted on the 2017 draft are still applicable in the 2020 version! If EMA's objective was to

codify practices appropriate for the 1970s and 1980s then they may have succeeded. We find it unconscionable for a major international regulatory agency to provide a current GMP document so backward in its thinking. A technical field of enterprises requires something beyond prescriptive, dogmatic standards that fail to acknowledge innovation; standards that are too rigid to accommodate further innovation are an obstacle to further improvement.

EMA provides no supportive rationale for its document's numerous positions, which align poorly with best industrial practices, nor has it established a means for open communication with the authors and others who have commented on the proposed Annex 1 revisions. Its refusal to adequately engage with those obliged to conform with the content is both inappropriate and discouraging. This document has massive implications for suppliers and manufacturers in a $10-trillion global industry and that input is vital.

Rather than spend inordinate amounts of time to comment on a document so lacking in scientific or process engineering awareness in a process so dismissive of external input we have chosen not to submit detailed comments. Instead we have summarized our major objections to the February 2020 draft below:

- Many Annex 1 requirements are based upon design and performance expectations derived from obsolescent practices heavily dependent upon manual operations. Their inclusion perpetuates the use of facility and equipment designs minimally suited for current use and wholly inappropriate for the future. This is how innovation is stifled.

- The Annex places undue emphasis on viable environmental monitoring, process simulation and sterility testing as a means of assessing system performance. These microbiological methods have limited sensitivity and inherently high variability. It is simply not possible for advanced technologies used for sterile product manufacturing to rely on measurement systems inadequate to assess their performance. With many newer technologies, these practices have no statistically valid utility. Mandating their inclusion is an obstacle to further performance improvement.

- There is a continued deference in the document to the conduct of microbial, chemical, and physical tests rather than to prospective validation of systems, processes, and products. Quality is a consequence of proper conceptual design and engineering. Quality is then maintained over the life cycle of the process by proper change management and statistical processing control. Over-emphasis on testing is antithetical to the principles of Quality Risk Management as espoused in ICH Q9 and other documents. This industry has long understood that it is not possible to test or monitor quality into a process. Nevertheless, the authors of EU Annex 1 persist in the misguided and erroneous belief that testing can prove what we have all learned it cannot.

- The sterilization content relies in part on antique dogma and lacks scientific justification. The origins of this content date to the 1970s and represent a poorly conceived means for sterilization process control based upon problems in a non-qualified sterilizer in a hospital setting. The included perspective is at odds with more recent peer reviewed content provided in USP chapters.

- Treatment of separative technologies is badly structured and largely equates minimally capable systems with the most advanced aseptic processing technologies currently available. Setting the bar so low as to render it invisible makes a mockery of the document's claims that it will enhance performance. This content actually serves to suppress further improvement in aseptic processing by severely denigrating the superior capabilities of its most evolved technologies. Annex 1 reflects a lack of understanding of the human being as the principal source of contamination and its authors seem unable to fully grasp the significance of removing the human contamination source from the critical environment.

- The inclusion of prescriptive "how to" content throughout the document restricts innovation by precluding the use of technological advances. Regulations should refrain from

imposing detailed instructions on manufacturing practices and instead rely on the expertise of the drug substance, drug product manufacturer and their suppliers for best practices. Limiting regulation to "what to" would acknowledge that the EMA authors lack adequate contemporary expertise to establish production processes, especially given the rate of technology improvement.

- The Quality by Design principles outlined in ICH Q9 are insufficiently advocated and in some cases ignored in Annex 1. Risk mitigation is inhibited when improvements must be aligned with outdated precepts. The document touts that it is "risk based" but does not demonstrate that in substance. Aseptic processing and sterilization are primarily aimed at mitigating risk of infectious disease arising from products, but this Annex 1 draft ignores the most effective microbial risk analysis tools, which are Hazard Analysis Critical Control Point regimens.

Collectively the authors of this response have more than 150 years of experience in the healthcare industry and have worked extensively with regulators, suppliers, and healthcare manufacturers on six continents. We have published articles, provided training programs and offered presentations on sterilization, industrial microbiology, sterility assurance, quality assurance and process validation to define and advance state-of-the-art practices. Our goals have always been to offer the soundest and most appropriate solutions to advance process performance, particularly in the areas of patient safety.

In addition to our work as consultants, we have worked extensively within the United States Pharmacopiea (USP) to help establish a body of standards that embrace practices of varying capability for sterile products without inhibiting further advances. We have included a list of the USP chapters that more appropriately define sterile product standards than those in the draft Annex 1.

We have made numerous publications specific to the 2017 and 2020 revisions of the Annex and these are listed below. These should be given due consideration. In addition, we have published extensively on relevant subjects of the Annex, and many of those listed below influenced our comments and opposition to the drafts.

These publications elucidate sterile product manufacturing outlining aspects of design, manufacture and analysis that are in sharp contrast to the dated concepts embodied in the draft Annex 1. We encourage EMA to reconsider advancing the current draft and develop one that does not denigrate the best available current day technologies, nor restrict future improvement.

USP CHAPTERS

<1229> Sterilization and subchapters <1229.1–1229.18>.

<1228> Depyrogenation and subchapters <1228.1–1228.5>.

<1211> Sterility Assurance.

<1116> Microbiological Control and Monitoring of Aseptic Processing Environments.

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REGULATORY GUIDANCE, STERILIZATION AND ASEPTIC PROCESSING

This section contains documents unrelated to each other but addressing subjects of importance related to pharmaceutical manufacturing and control. The commonality in them is their shared goal to elucidate areas where we believe potential improvements in practice are needed.

6

WHAT, NOT HOW

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INTRODUCTION

Effective regulation demands a carefully considered focus: it should be specific enough to convey meaning but not so specific that implementation is impractical, leads to excessive costs or jeopardizes the situation the regulation was meant to manage in the first place. The fable *Goldilocks and the Three Bears* shows the benefit of this middle ground (1). Good regulations should point to a goal to be achieved rather than detail how that goal is to be accomplished Doing so results in needed flexibility to adopt new and emerging technologies and to accommodate other situations that might not have been anticipated when the regulation was implemented. The pharmaceutical industry struggles with complying with disparate global regulatory approaches, driving up costs and in some cases adversely affecting product quality and patient safety.

The core expectations for the quality of drug products expressed in good manufacturing practice regulations (CGMP) relate to the attributes of identity, strength, quality, purity, and potency of the

drug product – the product that has been developed and approved to cure, mitigate, treat or prevent disease in the patient who will use the product. Each of these attributes speaks to *what* a drug product is required to possess, and these attributes are assured when the product is made in accordance with CGMP.

CGMP regulations describe the practices to be followed which support the quality expectations for the drug product; they expand the *what* and define the core practices to be followed in its manufacture. They do not, in general, prescribe the *how* of manufacture. The core CGMP documents of pharmaceutical manufacturing, FDA's 21 CFR 211 and EMA's EudraLex Volume 4, describe the *what* quite clearly and in general terms, affording maximum flexibility to the manufacturer with respect to the design, execution and control of the manufacturing process.

Conversely, in the United States the how of pharmaceutical manufacture is presented in various guidelines, which are not binding and do not have the force of law. These correspond to the individual EMA Annexes which are considered legal requirements, and therein lies a problem, i.e., regulations that specify how.

A BRIEF HISTORY

In the early 1960s, Thalidomide, a widely prescribed drug for morning sickness in pregnancy, was determined to have caused birth defects, leading to the 1962 Kefauver-Harris Amendment to the US Federal Food, Drug, and Cosmetic Act, which among other provisions authorized the US Food and Drug Administration to issue good manufacturing practice regulations for "manufacturing, packaging, or holding of finished pharmaceuticals." Those regulations were finalized in 1963 as 21 CFR 133 "Drugs; Current Good Manufacturing Practice in Manufacture, Processing, Packing, or Holding" (2).

The US CGMP regulations underwent a major revision in 1978 (3). Subsequent revisions to US and international regulations and guidance documents stressed the need for process validation and quality management systems to ensure pharmaceuticals and biopharmaceuticals are safe and effective.

THE US CGMP REGULATIONS SPECIFY WHAT, BUT NOT HOW

The core CGMP regulations do not define the means of manufacture to be followed. Given the breadth of products and processes utilized this is wholly appropriate. The product and processes used are unique to each other and imposition of a singular approach, given that diversity, is too restrictive. FDA's guidelines allow each manufacturer freedom to establish the individual methods for production of its products as it sees fit. FDA guidelines include the following disclaimer. "*This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulation ….*" This allows flexibility on the part of the producer to implement technologies that achieve comparable results with practices that might vary from those described in the guidelines.

EMA CGMP REGULATIONS INCLUDE BOTH WHAT AND HOW

EMA Eudralex Volume 4 aligns itself with FDA 21 CFR 211 regulation by addressing the general requirements for compliant manufacturing (4). The supportive Annexes resemble in many ways the guidelines in the US. Significantly, the Annexes are considered requirements and non- conformance to them is reason for disapproval of a site or rejection of materials (5). There is little, if any, tolerance on the part of EMA inspectors for alternatives and no clear path to relief.

WHAT WAS SUPPOSED TO HAPPEN?

Regardless of the exact legal status of FDA guidance and EMA annexes, one aspect of both is crystal clear – they endeavor to define the specific means by which a product is to be manufactured or processes are designed and controlled (6–8). While intended to aid manufacturers, guidance documents may restrict innovation, and

rooted in design principles from what are now essentially obsolete and antiquated approaches, they reduce the ability of firms to innovate. Railroads, automobiles, and airplanes were invented, and those technologies were developed and refined, decades before regulations were established to control their use. Surprisingly, that is exactly the scenario the pharmaceutical industry was intended to experience. In 2002, FDA launched a major initiative – *Pharmaceutical CGMP Initiative for the 21st Century – a Risk Based Approach*, which suggested simultaneous improvements in pharmaceutical manufacturing and regulation. It envisioned a future where advances in one would be supported by the other. The International Conference on Harmonization (ICH) outlined a similar stance in Q9 – Quality Risk Management (9). Quality by Design and a focused subset of it, Sterility by Design, were heralded as positive measures supporting improved quality for pharmaceutical products (10). The long-standing quality emphasis on sampling and analysis would be replaced by robustly developed and implemented processes relying upon sound science and engineering.

WHAT ACTUALLY OCCURRED

Nevertheless, on the same day that FDA published the final report on its risk-based approach to CGMP it issued the final version of its guidance on aseptic processing (11, 12). The aseptic processing guidance included a number of processing expectations based upon practices originally established in the 1950s! The guidance also flew in the face of the risk-based approach outlined in the *Pharmaceutical CGMP Initiative for the 21st Century*. Not only that, more than decade later, in December 2017, EMA took this to an entirely new level with its draft revision of Annex 1, Manufacture of Sterile Medicinal Products which employed a similar approach and expanded upon the number of numerical constraints on manufacturing practices for sterile products (13). The January 2020 draft revision of Annex 1 was largely organizational and many of the quantitative precepts from the prior draft went unchanged (14).

WHY DOES THIS MATTER?

While our industry is dependent upon innovation for the identification and development of new therapies, improvements in the means of their production are stifled by adherence to expectations derived directly from practices so antiquated they would have been considered non-compliant decades ago. Expectations intended for facilities heavily reliant on aseptically gowned personnel have no utility with automated filling systems within isolators. Air velocity, smoke studies, air change rates, active air sampling, recovery times and more may have had relevance in our operator-dependent past, but the new technologies in use should be subjected to controls appropriate to their design. Innovative technologies such as single-use systems, robotic manufacturing, lights-out aseptic operations, gloveless isolators and blow-fill-seal systems obviate the need for many of the monitoring and control systems specified in regulatory guidance documents. There is nothing magical or particularly beneficial about a requirement for HEPA-filtered air velocities of 90 ft./min. in manned cleanrooms or unidirectional airflow in closed isolators. Risk-based analysis and quality by design should inform needed production, control and product quality requirements. It is time for regulators to actually apply the principles they espoused in *Pharmaceutical Quality for the 21st Century – A Risk-Based Approach* and ICH Q9 and refrain from drafting guidance documents that do not comport with those principles.

CONCLUSION

Tell us what, not how. Regulation should never take precedence over good engineering and good manufacturing practices. Regulatory agencies often dictate requirements based on perceived or actual local conditions in an effort to minimize “risk.” Doing so often adds unneeded complexity, increases costs and results in difficult-to-control processes with potentially inconsistent output. It also severely restricts the ability to further improve performance by mandating “requirements” poorly suited to advancing technologies. As professionals, we have to continually press regulators to let us do

our jobs and design robust and validated systems that consistently produce the expected output, thereby maximizing product quality and patient safety. To all regulators out there, please tell us what, not how. We're the experts, not you.

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7

ADVANCING THE STATE OF ASEPTIC PROCESSING: LET'S GET SERIOUS

Phil DeSantis

Having spent over 50 years in the pharmaceutical industry, I have reached a state of frustration so great that I am almost at the point of doing something that I have never really considered … retiring. For the moment, I remain committed to my work and to the industry organizations that I actively support – PDA, for which I am Chair of the Science Advisory Board, and ISPE continue to do a great job representing industry interests and providing practical guidance on manufacturing issues. I also have a great respect for the regulators, FDA, EMA and their counterparts, for the job they do with regard to ensuring that the drugs they approve are safe and efficacious. On the other hand, there are things that disturb me so greatly that my commitment is cracking.

While science and technology are strongly rooted in the *laboratories* of both industry and government, they seem to take a back seat in that critical last phase that has a direct effect on the patient: *manufacturing*. I am so distressed by the state of drug manufacturing that I feel the need to write this post.

Generic drugs are in crisis, especially sterile generics. The drug shortage list continues to be a lengthy one. The cost of production

has been pressured by archaic requirements intended to control contamination and ensure quality, but that are often misplaced and no longer effective in achieving those goals. Generic manufacturers in developed nations, where regulations are strictly enforced and inspections are frequent and thorough, have been forced to close their doors. They have been unable to bear the cost of compliance and still remain profitable. Regrettably, the US and Europe have relinquished much of the responsibility to manufacture generics to geographies which appear more interested in low cost and fast profits than quality.

So, what is the solution?

In 2002 the US FDA announced *Pharmaceutical CGMPs for the 21st Century – A Risk-based Approach*. Can you imagine this was nearly two decades ago? The purpose of this initiative was nominally to modernize the FDA's regulation of pharmaceutical manufacturing and promote technology advancement. Moreover, the initiative encouraged a new culture of continuous improvement within the manufacturing segment of the industry. In the years following the launch of the program, the FDA has adopted its core principles, as follows:

- Risk-based orientation.
- Science-based policies.
- Integrated quality systems orientation.
- International cooperation.
- Strong public health protection.

Throughout the initiation, implementation, and continuation of this intended modern approach, the primary focus was always "to minimize the risks to public health associated with pharmaceutical product manufacturing." Europe and Japan followed suit with their own publications, as well as participation in the International Council on Harmonization Guidance for Industry *Q9 Quality Risk Management*.

About two years ago, I published in *Pharmaceutical Online Newsletter* a two-part article entitled *Facilities and Equipment Risk*

Management: A Quality Systems Approach. (I am, after all, an engineer. Facilities and equipment remain among my key interests.) In that original article I stressed three themes, which I called the Rules of Quality Risk Management. I'll list them here, but will not elaborate. I direct the reader to the original text at the following link:

https://www.pharmaceuticalonline.com/doc/facilities-and-equipment-risk-management-a-quality-systems-approach-0001

- First Rule of Quality Risk Management: "At the end of the day, all that really matters is the patient."

- Second Rule: "If everything is critical, then nothing is critical."

- Third Rule: "Dogma breeds ritual; ritual breeds waste".

To summarize that series, I borrowed from Lean Principles and suggest that *everything* you do must enhance the quality of your product. I thought that, considering my current state of mind and with two years of opportunity for regulators and industry to further advance the worthy cause of QRM, I would take another look. This time I have chosen a broader perspective toward manufacturing. Further, I have chosen to focus on manufacture of sterile products because (1) this continues to be a crisis topic with regard to the supply chain, (2) it arguably represents the greatest risks to patients and (3) it is the subject of current debate because the revision to EU GMP Annex 1 is currently under industry review.

From a QRM perspective, the state of sterile drug manufacturing continues to be dire. Short supply and contamination seem to be battling each other in some apocalyptic rugby scrum. The solution to neither seems within the realm of reality, as the attempts to eliminate the latter seem inevitably to exacerbate the former. Why should this be?

Recently, I have been spending, perhaps wasting, a huge amount of time helping to develop an industry response to the revision of Annex 1. That document is arguably the most important regulation dealing with the manufacture of sterile drugs. Its principles are

applied throughout the world. Will this long-awaited revision actually improve the quality of the world's sterile drug supply? Will it reduce contamination? Will it help to deal with drug shortages? In my opinion, not one bit.

Within the industry, trends are not nearly as encouraging as they should be. A 2020 ISPE survey of so-called "barrier" technologies indicates that among the manufacturers of filling lines surveyed, the number of traditional non-barrier lines specified for installation have steadily decreased over the preceding five-year period. The number of new barrier systems, however, are significantly weighted toward restricted access barrier systems (RABS) rather than isolators, with the latter accounting for less than 30% of all new installations. Based on previous surveys (2011, 2012 by ISPE) that percentage has not increased significantly. In 2012 isolators were estimated to be around 25% of all operating systems. I am discounting the value of RABS because they allow direct personnel access to the critical zone and do not provide the optimal level of contamination control that is attainable with an isolator. Sure, they are better than the non-barrier lines, but I cannot consider them good enough. Regulators express their agreement by continuing to require full aseptic garb and rigorous clean room design not required for true isolators.

So it remains that a large part of industry continues to rely on outdated and high risk aseptic processing methods, even though more advanced risk-mitigating technology has been available for more than 30 years. At least partially because of this, the authors of Annex 1, and regulators in general, continue to foster traditional means to compensate for these precarious manufacturing methods rather than to advance the use of technology, good design and best practices to ensure sterility. The methods employed have become virtual rituals and include intense environmental monitoring, frequent aseptic process simulations (media fills), airflow visualization studies ("smoke tests") and others.

Of course, these methods do not mitigate or control contamination in any way. They merely attempt to indirectly measure it, and not particularly effectively. This approach perpetuates a dogma that testing is an acceptable substitute for effective design. By continuing to overemphasize monitoring and testing, the burden on sterile

products manufacturers is likely to force even more plants to close their doors.

In the end, does it look like we are managing risk? Considering that aseptic processing presents the greatest contamination risk to patients, QRM appears to be just another slogan to be lauded, then forgotten.

So, what do we do as an industry? It seems pitiful that, for whatever reason, industry has failed to universally adopt more effective technologies to control contamination resulting in safer sterile drugs. On the other hand, the regulators must share substantial blame because of their historically luke-warm acceptance of emerging and even established advanced technologies. As an example, the regulations and field investigators continue to treat isolators as "little clean rooms", which they most definitely are not. They would serve the patient population so much better if they would abandon their efforts to fine tune outdated regulations and guidances. Let's leave Annex 1 and its related guidances as they are. They have been adequate for the last 10 years and should remain so for a few more. Take QRM seriously and really do something that mitigates risk effectively. Begin work on a deployment plan to eliminate traditional aseptic processing and all of its human intervention. Provide a transition period, say five or 10 years, to require the use of proven and emerging technologies (e.g., isolators, robotics, closed-vial filling, etc.) that will separate the product from the sources of contamination and achieve the ultimate goal once and for all.

What about RABS, that partial measure that doesn't quite make the QRM grade? There are, after all, a lot of RABS out there (the same surveys ball-park this at 25–30% of all lines). Considering the true application of QRM, these should all be replaced or, for those closed RABS that allow, converted to true isolators. There is no question in my mind that our engineers can develop a workable solution to this dilemma.

What about cost? We have become so concerned with cost that we have already driven the profit margin of sterile generics into the ground. Shortages continue to grow. Patients are without the drugs they need and much of what is available has earned a reputation of being below acceptable quality standards. Of course there will be

a short-term cost. Companies, governments, insurance companies and individual consumers need to be willing to bear this. The good news is that studies have shown that in the longer term isolators can actually reduce the operating cost of aseptic processing, and if the regulators practice their end of QRM, much of the monitoring and challenge testing associated with traditional aseptic processing can be eliminated.

We need to bite the proverbial bullet and do this now. Surely, it will be a hard transition, but at the end of the day, all that matters is the patient.

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8

REDECORATING THE DECISION TREE

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In March 2019, EMA issued the second official version of its Guidance document on the selection of sterilization methods for pharmaceutical materials (EMA, 2019). This document includes physical requirements which prescribe the expected process conditions to be used. The specifics vary from the earlier version, but the underlying principle is essentially unchanged – that regulatory defined conditions are appropriate in the selection of sterilization methods (EMA, 2020). The first referenced condition for steam sterilization links to the Ph. Eur. standard cycle of 121°C for 15 minutes throughout the load (Ph. Eur., 2008). These conditions first appeared in the global pharmacopeia 40 years ago in relation to the sterilization of laboratory media. The next tier of expected processes includes an F_0>8 minutes. This was identified as the minimum sterilization process by FDA in 1976! (FDA, 1976) Terminal sterilization practice in 2019 differs markedly from these ancient precepts. The United States Pharmacopeia (USP) acknowledged this in revisions to its sterilization related content that first appeared starting in 2013. (USP, 2913). The target in every sterilization process is the pre-sterilization bioburden, because its destruction is required to make the materials safe for use (Agalloco, 2017a). Recognition that reliable

bioburden destruction defines the process has been understood by large volume parenteral manufacturers globally for several decades as they successfully implemented terminal sterilization processes using conditions adapted to the specifics of the materials being sterilized and the expected most resistant bioburden isolate. The broader industry perspective is less enlightened and relies heavily on "overkill" processes and guidance found in EMA's decision tree. This leads to the use of cycles where "worst case" assumptions regarding bioburden resistance, bioburden population, biological indicator strain, biological indicator and copious safety factors are utilized. In combination these result in the use of sterilizing conditions that while certainly effective in assuring sterility, can cause adverse effects on the key quality attributes of the materials. One of the core concepts in the revised USP content was consideration of balancing the competing aspects of sterility and stability:

> "The selection of a particular sterilizing treatment and the details of its execution often represent a compromise between the conditions required to destroy or remove the bioburden to the desired level and the impact of the sterilization process on the materials being processed. Sterilization processes should be sufficiently robust for certainty of microbial inactivation while avoiding adverse consequences to material quality attributes." (USP, 2013)

The USP goal in its revision was to increase the use of terminal sterilization processes by eliminating the use of excessive conditions that while surely able to sterilize, were overly detrimental to product quality. The fixation on killing very high populations of resistant biological indicators (BIs) was believed to reduce the application of terminal sterilization. The emphasis in the Guidance is on physical measurements and prescribed limits for operating temperature, minimum F_0 and biological indicator D-values. The EMA Guidance does not adequately consider the importance of maintaining finished product quality in selecting the terminal sterilization process.

If the goal is advancing patient safety, the wider use of terminal sterilization needs to be better supported. The process constraints in the EMA Guidance are contrary to that objective. Section 4.1.1 of the EMA Guidance describes the use of processes operating at less than

110°C and delivering an F_0<8 minutes. Where these parameters are employed the filled units must be produced by aseptic processing. The Guidance identifies the process as "post-aseptic processing terminal heat treatments" something less than terminal sterilization.

The Guidance repeats, and reinforces, misconceptions that have plagued terminal sterilization for decades. Preferences for "overkill", the use of biological indicators with defined minimum resistance, minimum time-temperature and minimum F_0 are rooted in the belief that destruction of millions of highly resistant microorganisms is required for confidence in terminal sterilization. All of this might be well intended, but it leads away from the true objective – delivering safer medications to the patient.

The USP's sterilization content flips the narrative, by addressing the true target of the sterilization process – the pre-sterilization bioburden of the materials. The biological indicator's role is to confirm that the physical measurements are accurate assessments of the expected lethality during the validation effort. The BI is not present in any container administered to a patient. Conversely, every container has some potential bioburden present whose destruction in the terminal process must be assured. The sterilization process utilized must establish that each produced unit is safe for patients and should be based upon the population and resistance of the bioburden. This is central to USP's sterilization content, and when properly applied allows for flexibility in sterilizing conditions without arbitrary and irrelevant constraints. The importance of reliable information on the bioburden population and resistance cannot be overstressed.

Patient safety on an every lot basis is established by calculation of the Probability of a Non-Sterile Unit (PNSU) using data collected from bioburden samples from pre-sterilized containers.

> "Articles intended to be sterile must attain a $\leq 10^{-6}$ PNSU, i.e., less than or equal to 1 chance in 1 million that viable bioburden microorganisms are present. The PNSU can be determined from Equation 1.
>
> $$(1)\quad \log N_u = -F/D + \log N_0$$
>
> N_u = PNSU
> D = D-value of the natural bioburden
> F = F_0-value of the process (lethality)
> N_0 = bioburden population per container" (USP, 2013)

Why is this important? Simply put, it defines the process as that necessary to reliably destroy the bioburden present. It's easy to add safety margins to increase confidence in the process. The PNSU target can be altered to one chance in a billion; the bioburden population assumption set several logs above the typical maximum and a "worst case" bioburden resistance assumed. Bioburden resistance has been largely ignored in our obsession with the use of "overkill" and worst yet, "half-cycle" approaches to sterilization cycle development (Agalloco, 2007, 2016). The available historical data on bioburden resistance are limited, and reliable methods for its determination did not previously exist (Pflug, 2010; Agalloco, 2017b, c, d). The means for bioburden resistance determination are just now emerging in USP and these may serve to both increase the body of knowledge and instill greater confidence in terminal sterilization processes overall (Agalloco and Tidswell, 2018). The limited information on bioburden resistance available today foretells a future quite different from that attainable following the EMA Guidance. Recognition that a process need only provide time-temperatures (or kGy for radiation) to destroy minimally resistant bioburden to ensure patient safety can be realized with terminal sterilization at temperatures (or radiation dose) much lower than the EMA proscribed 110°C. In 2012, a collaboration of Japanese firms issued a guidance document on Terminal Sterilization similar to that of USP that focuses the sterilization process on bioburden destruction (MHLW, 2012). This echoes contemporary practice in Japan where a large number of products are terminally sterilized at F_0 values of less than eight minutes with some process with process targets less than F_0=2 (Sasaki, 2002). The products processed in this manner are produced using highly automated systems with minimal human intervention, but without the types of aseptic controls mandated by the EMA Guidance. With newer technologies, the ability to assure near-absolute bioburden control is assured without the accoutrements of manned aseptic processing. The methods used are consistent with the newly revised USP chapter on Sterility Assurance, which focuses on system design and procedural controls as the means to support effective microbial control for all types of products.

There should be no disagreement regarding a preference for terminal sterilization in the production of sterile materials. Realization of this must allow for greater use of terminal processes absent the artificial constraints of minimum time-temperature, F_0 or kGy to be demonstrated. In addition, the biological indicator must be recognized as a tool to measure the process, not to define its duration. Terminal processes must be able to consistently destroy the pre-sterilization bioburden, allowing for the use of less aggressive condition and simultaneously enabling wider use of terminal treatments. Prescriptive approaches as defined in the most recent EMA Decision Tree do not lend themselves to expanded use of terminal processes. A patient focused Decision Tree would have fewer branches. The first branch would allow for direct implementation of the Ph. Eur. standard cycle of 121°C for 15 minutes throughout the load only because it reduces the validation requirements. The only other branch would ask whether the product can use a validated sterilization method to assure a PNSU of less than 1 in 10^6 for the pre-sterilization bioburden. It's time to redecorate the tree!

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9

... AND THE AWARD FOR BEST PICTURE GOES TO ...

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THE WHY AND HOW OF SMOKE STUDIES

- To visualize air flow patterns in critical environments, under both static and dynamic conditions to check for the presence of "eddies", dead zones, and backflow of air that might result in the contamination of materials.

- By the introduction of "smoke" upstream in the air flow and visually observe its movement over critical surfaces.

THE WHAT OF SMOKE STUDIES

- Review of video images in critical environments to establish the appropriateness of the air flow patterns observed. Looking for:
  - "First air" – disruption of unidirectional flow
  - "Eddies" – suggesting retention of particles
  - "Dead zones" where particles might accumulate
  - Intervention created problems associated with the above (dynamic only)

WHAT DO SMOKE STUDIES SHOW?

- Identification of facility and equipment design and operation that may be inadequate to protect sterile materials and critical surfaces.

- Identification of interventional procedures that might result in the introduction of contamination.

SMOKE STUDY – STATIC CONDITIONS

Depicts impact of design on air patterns

SMOKE STUDY – DYNAMIC

Depicts impact of interventions on air patterns

WHAT'S THE PROBLEM – 1

- We've all heard the adage – "beauty is in the eyes of the beholder." It couldn't be truer than with smoke studies.
- Smoke studies have a singular perspective, and while they can play a useful role in the evaluation of an aseptic process design and operating practices, they will always be subjective assessments.
- There's simply "too much" to see.

WHAT'S THE PROBLEM – 2

- Firms invest millions in their facilities, equipment, processing. and procedures for aseptic processing.
- It must be done properly with attention to many details in order to minimize contamination.
- Personnel must be initially and constantly retrained in aseptic practices.
- Judging the correctness of everything from 30 seconds of video simply isn't justifiable.

HOW TO SUCCEED – 1

- The facilities, equipment and procedures used for aseptic processing require fastidious attention to detail to assure success.
- Sterility by Design concepts outline the elements necessary.
- These must be considered in depth, established and maintained to provide the desired performance.

HOW TO SUCCEED – 2

- Perform studies that simulate the static state and each of expected interventions performed in the critical zone.
- Airflow/curtains/barriers/access and especially procedures should be carefully reviewed and adjusted to minimize air passage over the operator and onto sterile objects and critical zones.
- Follow "first air" principles at all times.
- If something isn't correct, make changes and review again until satisfied.

HOW TO SUCCEED – 3

- For "dynamic" studies make sure the "actors" are well rehearsed.
- They must be trained and proficient in all interventions and perform them as described in procedures.
- If physical changes are made to improve the "take" consider the potential impact on the "as built" videos and reshoot them if necessary.

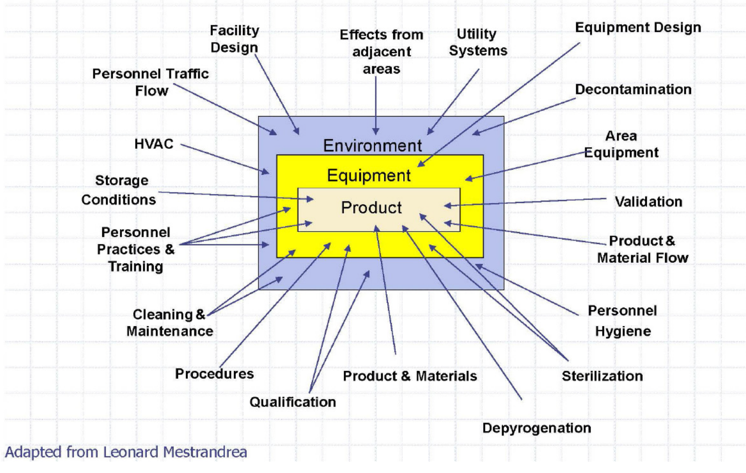
HOW TO SUCCEED – 4

- Think like a movie director with respect to camera angles, lighting, smoke density, etc.
- Understand that great scenes are rarely made in a single take.
- This isn't editing, it's searching for the correct way to show the "story".
- Since the interpretation of results is always subjective, be conservative and review the video until multiple viewers agree on the image that captures the "story" best.

WILL YOU WIN "BEST PICTURE"?

- That decision is always a subjective one.
- We all see the same images but will almost always have differing opinions about what we've just seen.
- If the facility, equipment and process design is correct, you're halfway there.
- Filming your story carefully to represent it clearly offers the greatest potential to win the "Best Picture" award.
- Respect that any future "audiences" will each have their own opinions.

INFLUENCES ON STERILE PRODUCTS



TELL THE WHOLE STORY

- Success in aseptic processing derives from a myriad of successful decisions in the design of facilities, equipment and procedures.
- It's only a good as the "weakest link".
- Smoke studies are just another means to assess the suitability of your designs. They are a piece of the puzzle. Make sure the audience sees the entire picture!

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USP (2018) USP <1211> Sterility Assurance. United States Pharmacopeial Convention, USP 41-NF 36, supplement 2.

POSTSCRIPT

- My "Best Picture" choice was "Marriage Story".
- What was yours?
- Which picture won? Mine, yours or something else?
- I think we must all understand by now how it's extremely subjective and that's not going to change.

10

CUTTING THINGS IN HALF

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THE PROBLEM STATEMENT

- Environmental concerns related to ETO emissions have resulted in closure of a major contract sterilization site, delayed a site startup and operations at existing contract sites may be threatened.

- Reductions in ETO capacity may result in drug and device shortages.

OVERVIEW OF ETHYLENE OXIDE STERILIZATION

- Process challenges:
  - Multiple variables (critical parameters) to address
  - Long turnaround times
  - Highly flammable and explosive
  - Carcinogen

- Emission of ethylene oxide (EtO) gas resulting from sterilization is an identified concern:
  - The Environmental Protection Agency identifies ethylene oxide as a hazardous air pollutant
  - To date, we are aware of one industry EtO sterilization facility that has been closed on the basis of ethylene oxide emissions.
  - There are concerns associated with manufacturing and processing of devices historically sterilized with EtO.

FDA Webinar, October 25, 2019

MEDICAL DEVICE PUBLIC HEALTH IMPACT

- Unavailability of EtO as an industrial sterilant for medical devices is a concern.
  - The effectiveness of the technology for broad spectrum antimicrobial sterilization is well established.
  - The high throughput/low cost of this technology allows for large sterilization capacity which guarantees the supply chain of medical devices sterilized using the technology is preserved.
  - EtO is the only acceptable sterilization method for a large number of delicate, complex, and sophisticated medical devices manufactured with sensitive materials and removal of EtO as an option could lead to device shortages.

FDA Webinar, October 25, 2019

FDA'S ACTIONS

- Alerting industry to the potential for shortages.
- Expediting site change requests in the event of site closures.
- Innovation initiatives for:
  - Alternative sterilization methods
  - Reductions in ETO emissions

THE ELEPHANT IN THE ROOM

Sterilization using the overkill method (ISO 11135, Annex B) reduces available capacity.

> "Half-cycle approach: a total of three consecutive experiments resulting in total inactivation of the biological indicators (with a population of not less than 10^6 and, where appropriate, placed within a PCD) shall be performed in order to confirm the minimum exposure time. The specified exposure time for the sterilization process shall be at least double this minimum time."

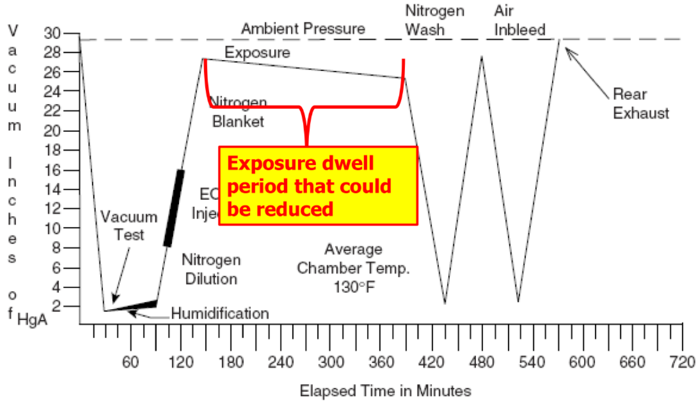
THE "HALF-CYCLE" METHOD – 1

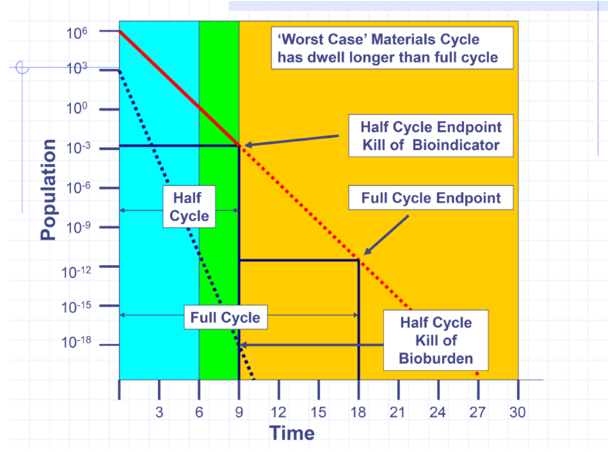
- This validation method is extremely conservative and extends the dwell period duration well beyond "overkill" as practiced with other means of sterilization.
- Essentially a double "overkill" cycle.
- Ignores the bioburden entirely and arbitrarily doubles the exposure time for routine cycles from the time shown effective for killing the biological indicator during validation.

THE "HALF-CYCLE" METHOD – 2

- Relies on temperature, RH and BIs, but measurement of ETO concentration is not required.
- Is far more conservative than "overkill" cycles used for validation of other sterilization methods. The "half-cycle" is already "overkill".
- Initially an expedient means of validation that predates real-time concentration measurements.

TYPICAL ETO CYCLE





HALF-CYCLE DIAGRAM (SEE ABOVE)

- Note how that at the end of the "half-cycle" dwell the bioburden PNSU is NMT 10^{-18}. That's well beyond the minimum expectations for an "overkill" sterilization process.

- The bioburden resistance to EtO may be substantially less than that depicted in which case the PNSU would be even lower.

EXAMPLES OF "HALF-CYCLE" APPROACH

	Concentration	RH	Temperature	Time
	PPM	%	°C	Hours
"Half Cycle" Process	600 650	70 75	30 35	4
"Full Cycle" Process	600 650	70 75	30 35	8
Material Evaluation Cycle	600 650	7 75	30 35	>8

A MORE SCIENTIFIC APPROACH

THE BRACKETING APPROACH

- The use of "worst case" cycle parameters that evaluate the effect of more than just time on cycle lethality.
  - "Worst case" sterilization cycle – using lower concentration, RH, humidity and less time where complete kill is achieved.
  - "Worst case" material cycle – using higher concentration, RH, humidity and more time where material effects are evaluated.

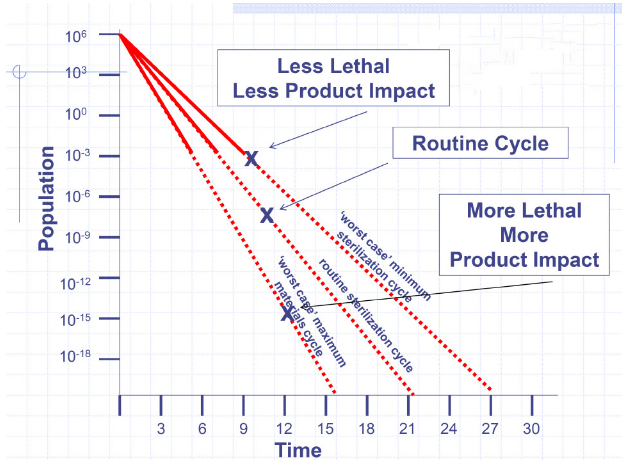
- These bracket the routine cycle parameters to ensure process robustness.

BRACKETING APPROACH FOR ETO

- Establish kill of BI using the same means as for "half-cycle". This is the "worst case" for kill – the minimum sterilization cycle.

- Increase cycle duration, gas concentration, RH and temperature to increase lethality for routine use. Twice the dwell isn't necessary.

- Further increase cycle duration, gas concentration, RH and temperature to confirm product compatibility. This is the "worst case" for material impact – the maximum materials cycle.



EX: BRACKETING APPROACH

	Concentration	RH	Temperature	Time
	PPM	%	°C	Hours
"Worst case" sterilization	600 650	70 75	30 35	4
Routine process	650 700	75 80	35 40	6
"Worst case" material effect	700 750	80 85	40 45	8

SOLVING THE PROBLEM

- Abandoning the "half-cycle" method as an anachronism of a long-gone era when validation didn't exist can relieve the capacity crunch. There's two paths forward:
  - Accepting the "half-cycle" segment for what it is: an "overkill" sterilization process.
  - Using a bracketing approach with the "half-cycle" as the lower edge of the bracket.
- Both paths leverage the existing "half-cycle" validation data as the baseline for implementation.

WHAT'S HOLDING THINGS BACK?

- Tradition – as the "half cycle" has been used for more than 60 years.
- Caution – it reduces the dwell period so it could be construed as less robust.
- Filings – submissions describing EtO sterilization almost universally cite the "half- cycle" method.
- Expense – some investment may be required to add concentration measurement to existing systems.

WHAT ARE THE BENEFITS?

- Reductions in process dwell periods will increase equipment utilization. Increased capacity might exceed 20%.
- If the bracketing method is used, process robustness is improved.
- We may be able to avoid shortages of important drugs and medical devices due to inadequate capacity across the industry.

11

DÉJÀ VU, ALL OVER AGAIN!

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MID-1970S

- Separate incidents with respect to sterility failures in terminally sterilized products in the USA and UK resulted in many deaths.

- The most significant outcome from a manufacturing perspective was the emergence of validation as a means of ensuring the reliability of processes. (Chapman, 1991)

FDA DEFINITION OF VALIDATION

"Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes." FDA, Guideline on General Principles of Process Validation, 1987.

THE ESSENCE OF VALIDATION

- End-product test methods were recognized as being inadequate to assure essential quality attributes due to statistical limitations in sampling and inadequate assay capabilities.

- Process control affords a measure of confidence in process outcomes unattainable from sampling and testing alone.

- This is implicit in CGMP regulations and is why process / product and system validation are critical to quality.

THE 1980S

- Validation programs were developed to embrace processes, methods, and systems of various types to independently ensure the reliability of output in a manner that testing alone could not.

- Industry performance improved markedly as validation-driven expectations reduced process variability in many areas.

VALIDATION IS CRUCIAL

- The sophistication of present-day equipment, systems and methods is such that their validation is required to ensure the desired outcome is achieved.

- No amount of end-product testing can provide the same confidence.

WHAT'S BEEN FORGOTTEN/IGNORED

- You can't test or monitor quality into a product.
- Quality by Design is sometimes the only means to ensure consistent results.
- This is particularly true where sampling and test methods lack sufficient statistical weight or analytical capability.
- Regulatory authorities do not recognize the inappropriateness of their expectations.

EXPECTATIONS FOR PERFECTION – FDA

- A single anomalous result requires investigation/remediation.
- Air monitoring samples of critical areas should never yield microbiological contaminants.
- Requirements for sterility of "critical" surfaces.
- A belief in precise microbial trends stemming from an unrealistic expectation of assay precision, limit of detection and limit of quantitation.
- Believes in "false negatives" in EM, but ignores the potential for "false positives."
- Routine identification of EM isolates to the species level.
- A mistaken belief in the value of EM resampling.
- No visible particles in finished drug products.
- No mold/yeasts in process environments.

EXPECTATIONS FOR PERFECTION – EMA

- Un-crimped containers maintained under Grade A.
- Containers closed by fusion subject to 100% integrity testing.
- Pre-use post-sterilization filter integrity tests are expected.
- Personnel monitoring post-interventions.
- Grade B throughout the aseptic core.
- Continuous monitoring of environments.
- 5 μm particles linked to microbial contamination.

THE PERILS OF PERFECTION

- More sampling -> more interventions
- More interventions -> more contamination
- More contamination -> more patient risk
- "The "perfect" intervention is the one that is not required." (Agalloco, 2005).

WHERE TESTING FAILS

- Tests that seek to establish absence or a negative absolute are inherently inadequate.
- These include:
  - Sterility testing
  - Absence of a particular microbial species/strain
  - Absence of particulate matter
  - Absence of impurities
  - Environmental monitoring in ISO 5 (Madsen and Agalloco, 2019)

WHERE TESTING WORKS

- When sampling sizes/data assembly is statistically significant.
- When validated assay methods have the capability to properly evaluate a quality attribute.
- When sampling cannot adversely impact product safety.
- When the gathering of data is not detrimental to the process outcome.

  "Many of the things you can count, don't count. Many of the things you can't count, really count." Sign in Albert Einstein's office

WHAT MUST CHANGE – 1

- Where the goal is extremely low defect levels, e.g., 6-sigma, emphasis must shift to the design and control of the process.
- Quality by Design in conjunction with superb process implementation and statistical process control are the only path forward.
- It is impossible to test or prove "perfection", however processes can be made sufficiently robust that we can rely on their performance.

WHAT MUST CHANGE – 2

- Regulatory emphasis must shift from testing to validated in-process control elements.
- Increased sampling and testing will not result in better process performance or improved product quality.
- Refinements in process design and control provide the only practical means for performance improvement.

WHAT HAS ALREADY CHANGED

- USP <1211> *Sterility Assurance* and <1115> *Bioburden Control of Non-sterile Drug Substances and Products* outline recommended practices focused on process design/control as opposed to sampling and analysis.

- These chapters are consistent with the general CGMP concepts in global regulations.

USP 39, 2014 & USP 43, 2019

THE IMPORTANCE OF VALIDATION

- Validation was the answer to critical product failures in the 1970s.

- While performance expectations have been made increasingly restrictive, the inability of end-product testing to establish absence is unchanged.

- Validation as a means of establishing process robustness has always been the only reliable means to assure product quality.

"Those who cannot remember the past are condemned to repeat it."
George Santayana, 1906

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OBSERVATIONS ON LOW ENDOTOXIN RECOVERY AND REGULATORY OVERSIGHT

We began our cooperative efforts with the subject of Low Endotoxin Recovery. The assembled collection presents our views on this subject where we believe the underlying science was largely ignored in a rush to regulate and publish.

12

THE FALLACY OF LOW ENDOTOXIN RECOVERY

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INTRODUCTION

Responding to reports of failure to detect spiked endotoxin in some biological drug products (Chen and Vinther, 2013) and FDA's request (Hughes, 2015) that companies conduct endotoxin spike/hold studies on biological products to determine whether endotoxin reference standard added to product formulations could be detected using USP <85> Bacterial Endotoxins Test (BET), the Parenteral Drug Association (PDA) published Technical Report No. 82 Low Endotoxin Recovery (TR 82) (PDA, 2019). The foreword to TR 82 contains the following statement:

> "... the PDA task force commissioned with this technical report went to the greatest lengths possible to present as complete a picture of the

> current LER situation. This includes the historical and mechanistic aspects of the endotoxin measurement challenges, as well as a standard protocol for developing product-specific hold studies, supported and informed by actual industry case studies ... PDA believes it is vitally important to make this information available to further the scientific dialog and progress in this area and remains committed to revising and updating this material as new discoveries and conclusions are made."

In the introduction to TR 82, the writers state:

> "The failure to recover spiked endotoxins from finished drug products suggested that endotoxin contamination from the manufacturing processes may not be detected at release, thus, pyrogenic products could be distributed for commercial use in patients. This task force, however, searched peer-reviewed, scientific literature, public data on recalls and adverse events, and available copies of the Centers for Disease Control Morbidity and Mortality Weekly Reports and found no instances where a bacterial endotoxin test (BET) failed to detect pyrogenic levels of endotoxin in samples of products released to the market ... Hence, published historical data provide no direct evidence of LAL failing to detect pyrogenic products."

This leads to the conclusion that low endotoxin recovery (LER), as such, is a misnomer – the issue is not LER, but low endotoxin standard spike recovery (LESSR).

TR 82 reports no instances where a bacterial endotoxin test (BET) failed to detect pyrogenic levels of endotoxin in samples of products released to the market; there is no risk to patient safety from non-detected endotoxin. TR 82 then goes on for over 100 pages discussing a phenomenon, LER, that doesn't exist. It is essentially describing LESSR, an interesting phenomenon, but one that does not affect product safety or public health.

DISCUSSION

In 2012 FDA issued guidance (FDA, 2012) on pyrogen and endotoxins testing addressing "... those issues that may be subject to misinterpretation and are not covered in compendial procedures

or in currently available guidance documents." The introduction of TR 82 reported that the technical report team members didn't find evidence that bacterial endotoxin testing failed to detect pyrogenic levels of endotoxin in samples of products released to the market.

The major concerns with LER, or more accurately LESSR, are:

- LER has been defined as the loss of detectable endotoxin activity over time in certain formulations containing chelators and surfactants commonly used in some biological and therapeutic protein products. LER is then a diminution of recovery over time and unlike the compendial BET assay the FDA spike recovery is done without product dilution. As an industry we have known for 40 years that interferences have occurred between product formulations and the BET. In some early industry studies over 70% of products inhibited the test and some 4% of products tested were incompatible (Twohy et al., 1984). Industry made the LAL test work despite inhibition using both chemical/biochemical countermeasures and dilution provided in the BET compendial chapter. In many cases dilution was all that was needed.

- Chelators were understood to have an inhibitory effect on the BET. It has been known from the outset that the enzymatic pathway on which the LAL test depends requires the presence of divalent cations. Low concentrations of divalent cations can impact the conformation of endotoxins and the LPS used as a calibration standard and their ability to be recognized by cell surface receptors. Chelating formulations of therapeutic proteins have been tested since the mid-1980s and the authors of TR 82 couldn't find a single adverse issue to report. It is absurd to think detection of endotoxin in these products is now or ever has been a problem.

- Reports came soon after the LER issue began to gain traction that it could be overcome by the use of what was called naturally occurring endotoxin (NOE). The Control Standard Endotoxins (CSE) and the Reference Standard Laboratories (including FDA's own laboratories) used NOE before the acronym LER

arose. There is no legitimate reason to suggest that any NOE preparation standardized against a RSE, and which meets the BET requirements for linearity, should not be used without regulatory question.

It is a given that regulators will regulate, and it is prudent that they do so to ensure patient safety. Committees and task forces are often initiated to address a problem, but that problem must be concretely demonstrated to exist. Furthermore, if a problem is objectively proven to exist, through well-designed experiments with proper positive and negative controls, the resolution of the problem must never be pre-determined. The PDA task force found that there is not now and never has been a public health crisis associated with LER. They wrote an entire report, TR 82, on LESSR, but inappropriately called it LER. LESSR has been known for decades, but LER is not settled science because there isn't evidence that it actually exists. Data, not emotion, assumption, dogma or ego is how the objective truth should be reached.

CONCLUSION

As indicated in the foreword to TR 82, PDA is committed to revising and updating the material as new discoveries and conclusions are made. It is time to honor that commitment.

FDA should formally revise its position on pyrogen and endotoxins testing to reflect the scientifically supported conclusion that the issue of LER is one of LESSR. There is no public health crisis associated with endotoxin testing.

There are no changes required to the compendial BET as a consequence of the presumed discovery of LESSR. It is a known phenomenon that the global industry has managed without hysteria for decades. There is no patient safety risk, and no justification for the extensive recovery studies mandated by FDA. Any BET revision would be in the hands of the entire community of stakeholders, as it should have been from the start.

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13

LER – FACT OR FALLACY?

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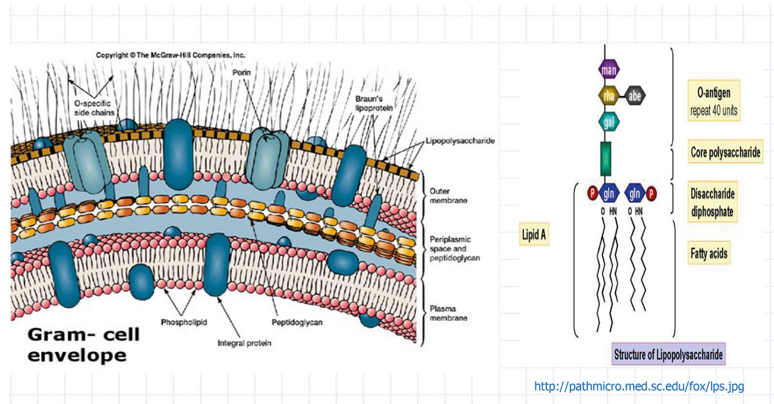
ENDOTOXIN: CELL WALL FRAGMENTS OF GRAM-MICROBES



Endotoxin: a pyrogenic (fever inducing) substance (e.g., lipopoly-saccharide) present in the bacterial cell wall of Gram-negative microorganisms. Endotoxin reactions range from fever to death.

OVERVIEW

- LER studies are an FDA imposed constraint on the biopharmaceutical industry lacking scientific merit.
- It describes a long known weakness in the analytical methodology.
- There has been and is no patient safety risk.
- Nevertheless, in the interim firms should comply with FDA expectations for LER testing until it is rescinded.

FDA IN A RECENT BLA

> "The following product testing and method validation information should be provided in the appropriate sections of Module 3.2.P: Certain formulations have been reported to interfere with endotoxin recoverability in the USP LAL test methods over time. The effect of hold time on endotoxin recovery should be assessed by spiking a known amount of standard endotoxin (CSE or RSE) into undiluted drug product and then testing for recoverable endotoxin over time."

LAL TESTING

- Bacterial 2.6.14 Endotoxin Test – USP <85>/ Ph. Eur.
- Accepted as an alternative to the Rabbit Pyrogen test for drug product release.
- Detection based on LAL clotting reaction.

- Gel Clot, Kinetic Colorimetric and/or Kinetic Turbidimetric Methods.
- Highly sensitive/rapid for Gram(–) bacterial endotoxin detection.
- Less sensitive or insensitive for other non-endotoxin pyrogens.
- Subject to interference by many factors.
- Some products simply cannot be assayed.

FACTORS INTERFERING WITH LAL TEST

- **Most substances, at concentrations used in pharmaceutical preparations, inhibit or enhance the LAL Test.**
- These substances/factors might give false-positive or false-negative results.
- Dilution, dialysis, addition of detergents (Pyrosperse®), and ultrafiltration have been described to overcome disturbing factors.
- Product dilutions within the calculated MVD or the use of endotoxin dispersing agents were found useful in reducing or eliminating inhibition.

Twohy et al. (1984)

- Assay interference can be caused by product-related physical or chemical factors
  - Physical inhibitors
    - Ionic
    - Adsorption or sequestration (of endotoxin)
    - Viscosity

- Chemical inhibitors
  - Chelation
  - Protein denaturation
  - pH perturbation

- Methods will be affected to varying degrees

LOW ENDOTOXIN RECOVERY

- Underestimation of lipopolysaccharide spike recovery in certain formulations of biological drugs when using LAL test methods.

- Observed after spiking lipopolysaccharide into undiluted protein biological products formulated with (polysorbate + citrate) or (polysorbate + phosphate buffers).

- Dependent on storage temperature, duration of storage and type of endotoxin spiked in undiluted product.

Chen and Vinther (2013)

SPIKE FOR RECOVERY STUDIES

- What is the appropriate surrogate to use?

- LPS – cannot/does not exist naturally.

- NOE – represents potential contaminants.
  - Standardized natural endotoxin for analysis

- Natural – what might actually be there.
  - Source is unknown
  - Potency is unknown
  - EU/ng is unknown
  - Can be a mixture of different endotoxins

Platco (2015)

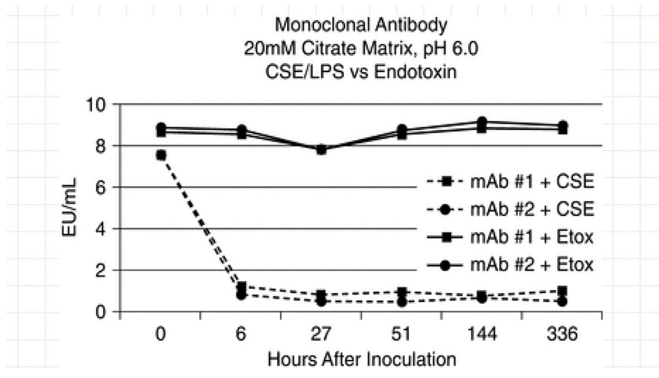
LER IS REALLY LESSR

- What was termed "low endotoxin recovery" was the inability to detect RSE/CSE in hold time studies in formulations containing chelators and surfactants.

- RSE/CSE are purified materials and differ markedly from NOE.

- LER is actually LESSR, because it is low endotoxin standard phenomena known spike recovery, a since the mid-1980s and easily accommodated by the <85>BET.

RECOVERY FROM mAB PREPARATIONS



Bolden et al. (2015)

IS LER CLINICALLY RELEVANT?

- For existing commercial products (some 10–15 years old), there have not been any significant clinical signals that can be related directly to LER.

- In the US, "Outbreak" type product recalls related to bacterial endotoxins have almost always been related to manufacturing or testing deviations.

CONCLUSIONS

- Products tested by USP <85> have been and continue to be completely safe.
- The current USP <85> BET methods fully assure patient safety.
- Current acceptance criteria are reasonable, prudent and conservative.
- No changes in industry production and test methods are needed.
- Endotoxin hold time studies for new biologics serve no worthwhile purpose.

NEEDED ACTIONS

- Recognition that the so called LER "problem" doesn't really exist. It's a known analytical issue that doesn't impact patient safety.
- Retraction of PDA TR# 82 as supporting an unnecessary "requirement" that serves no useful purpose.
- Withdrawal of FDA "informal" expectations for LER studies in BLA's and elsewhere.

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14

LER DECONSTRUCTED

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- Q3 … "Firms should establish procedures for storing and handling (which includes product mixing) samples for bacterial endotoxins analysis using laboratory data that demonstrate the stability of assayable endotoxins content." FDA, CDER, Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers, June 2012

- This expectation seeks confirmation of BET method sensitivity over an undefined time period.

The very next sentence is …

- "Protocols should consider the source of endotoxins used in the study, bearing in mind that purified bacterial endotoxins might react differently from native sources of endotoxins." FDA, CDER, Guidance for Industry – Pyrogen and Endotoxins Testing: Questions and Answers, June 2012.

- Results with Control Standard Endotoxin (CSE) or Reference Standard Endotoxin (CSE or RSE) are thus acknowledged to exhibit potentially different behavior from naturally occurring endotoxins (NOE).

THE CHEN-VINTHER EXPERIMENT

- Reports that various monoclonal antibodies formulations with divalent-chelating buffers and polysorbate prevented the recovery of LPS in hold time challenge studies (Chen and Vinther, 2013).

- The inability to recover Westphal extracted and purified LPS (CSE) in this type of formulation is a known phenomenon (Ribi et al., 1966; Twohy et al., 1984).

USP <85> BACTERIAL ENDOTOXIN TEST

- "Standard Endotoxin Solutions – After mixing the Standard Endotoxin Stock Solution vigorously, prepare appropriate serial dilutions of Standard Endotoxin Solution, using Water for BET. Use dilutions as soon as possible to avoid loss of activity ..." USP, <85> Bacterial Endotoxin Test, USP 39-NF 34. 2016.

- The globally harmonized <85> includes cautions regarding potential loss of LPS/CSE endotoxin activity over time even without exposure to any formulation.

- "Sample Solutions – Prepare the Sample Solutions by dissolving or diluting drugs, … using Water for BET. Some substances or preparations may be more appropriately dissolved, diluted, or extracted in other aqueous solutions. If necessary, adjust the pH of the solution to be examined …" USP <85> Bacterial Endotoxin Test, USP 39-NF 34. 2016.

- Dilution and pH adjustment of drug solutions is an accepted (and often necessary) practice for the <85> methods (Twohy et al., 1984).

WHAT IS LER?

- LER was termed Low Endotoxin Recovery by Chen and Vinther.

- This statement is not accurate.

- What could not be recovered was LPS/CSE which is a purified endotoxin used in the <85> method.

- LPS/CSE is a reference standard analyte used in USP <85>.

- CSE is a lipopolysaccharide that does not exist in its highly purified state in nature. As such it could never be a contaminant in a formulated product.

- The stability of LPS in endotoxin testing of a product is a laboratory analytical problem; not a patient safety concern.

WHAT IS NATIVE ENDOTOXIN?

- Also described as Naturally Occurring Endotoxin (NOE), or euphemistically as "dead bug parts."

- Endotoxin that might be introduced into parenteral formulations from Gram-negative microorganisms in water used in formulation, cleaning, components, active pharmaceutical ingredient or raw materials.

- It would be derived from the cell walls of Gram-negative microorganisms.
- Endotoxin is controlled by adherence to CGMP in the design, validation and process control in the production of injectable products.

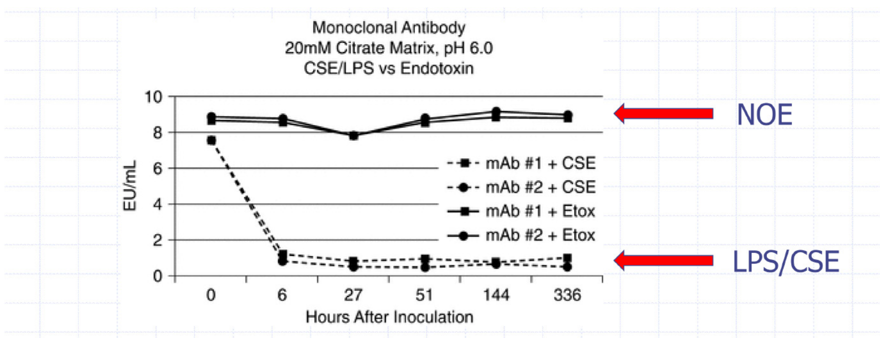
LPS/CSE COMPARED TO NOE

Purified LPS	Native Endotoxins
Biologically active portion is Lipid A that is stripped of its natural associated cell membrane components	Biologically active portion is Lipid A existing in a natural state that is surrounded by components that are part of native Gram negative outer membrane
Extracted, usually by a hot phenol extraction (Westphal) and further purified. It is ultimately formulated with chemicals that are not native to the cell membrane, usually polyethylene glycol and a sugar, prior to lyophilization	Exist as naturally generated and free floating outer membrane fragments and vesicles (blebs). LPS is embedded in or associated with cell membrane components (proteins, porin, phospholipids and lipoprotein). There is no extraction, no purification, and no further chemical formulation
LPS molecules form aggregates in aqueous solution, with the extent of the aggregation dependent on the formulation of the matrix	Natives do not form aggregates in the same way as LPS since native LPS is embedded within cell membranes of vesicles
Readily adsorbs to surfaces	Does not readily adsorb to surfaces
Does not exist in nature and therefore cannot and does not contaminate parenteral products	Native endotoxins can be natural contaminants in water systems and raw materials, particularly those of natural origin. If not controlled, these natural contaminants can find their way into parenteral products

McCullough et al. (2016)

NOE DOESN'T BEHAVE LIKE LPS/CSE

- When NOE is spiked into product formulations, there is no loss of endotoxin over time.



Bolden et al. (2015)

USP'S <1228> DEPYROGENATION CONTENT

- This chapter recognized the limitations of using LPS/CSE in challenges of depyrogenation processes.

- It was understood that LPS/CSE differ significantly from naturally occurring endotoxins such that LPS challenge studies could not support depyrogenation process efficacy.

- The substantial chemical and physical differences between LPS/CSE and NOE are such that destructive and removal processes that worked with LPS/CSE could not support comparable performance with NOE.

- This is wholly analogous to LER with LPS/CSE.

USP, <1228> Depyrogenation, USP 42-NF 37, 2019.

NOE DOESN'T BEHAVE LIKE LPS/CSE

- Chen's inability to detect LPS/CSE after exposure to mAb formulations should have not been a surprise.

- Bolden et al. and others have demonstrated that NOE exposed to similar mAb formulations is unchanged and fully detectable using <85> methods.

- So FDA's Endotoxin Q&A would be satisfied using a NOE challenge of the firm's choosing.

CIRCLING BACK TO THE BEGINNING

- If the LER concern is that endotoxin might not be detectable over time, it stands to reason that the concern be directed at an endotoxin that might *actually* be present.

- The inability to recover CSE is a laboratory method phenomenon that does not directly impact the patient. That is certainly true when the reference standard is known to be susceptible to the exact concern the stability study is intended to show.

- The most appropriate stability challenge would be one that more closely resembles what the patient must be protected from, and not a contaminant that would never be present.

- A NOE is the most logical choice.

NEXT STEPS – 1

- FDA's endotoxin stability concern should be addressed using an appropriate NOE challenge.

- The hold study required by FDA fails to establish that the BET assay is unable to detect endotoxin in product.

- The NOE to be utilized in recovery studies should be selected from natural endotoxins potentially present in the formulation.
- This approach addresses potential patient risk in a manner that LPS/CSE challenges cannot.

NEXT STEPS – 2

- The term "LER" should be abandoned forever as a misnomer.
- FDA's endotoxin Q&A should be understood as requiring challenge with a NOE.
- The NOE's potency should be calibrated against LPS/CSE.
- PDA's TR #82 should be revised to reflect the scientific realities resulting from the substantial biochemical differences between the reference standard (LPS/CSE) and native endotoxins (NOE).

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