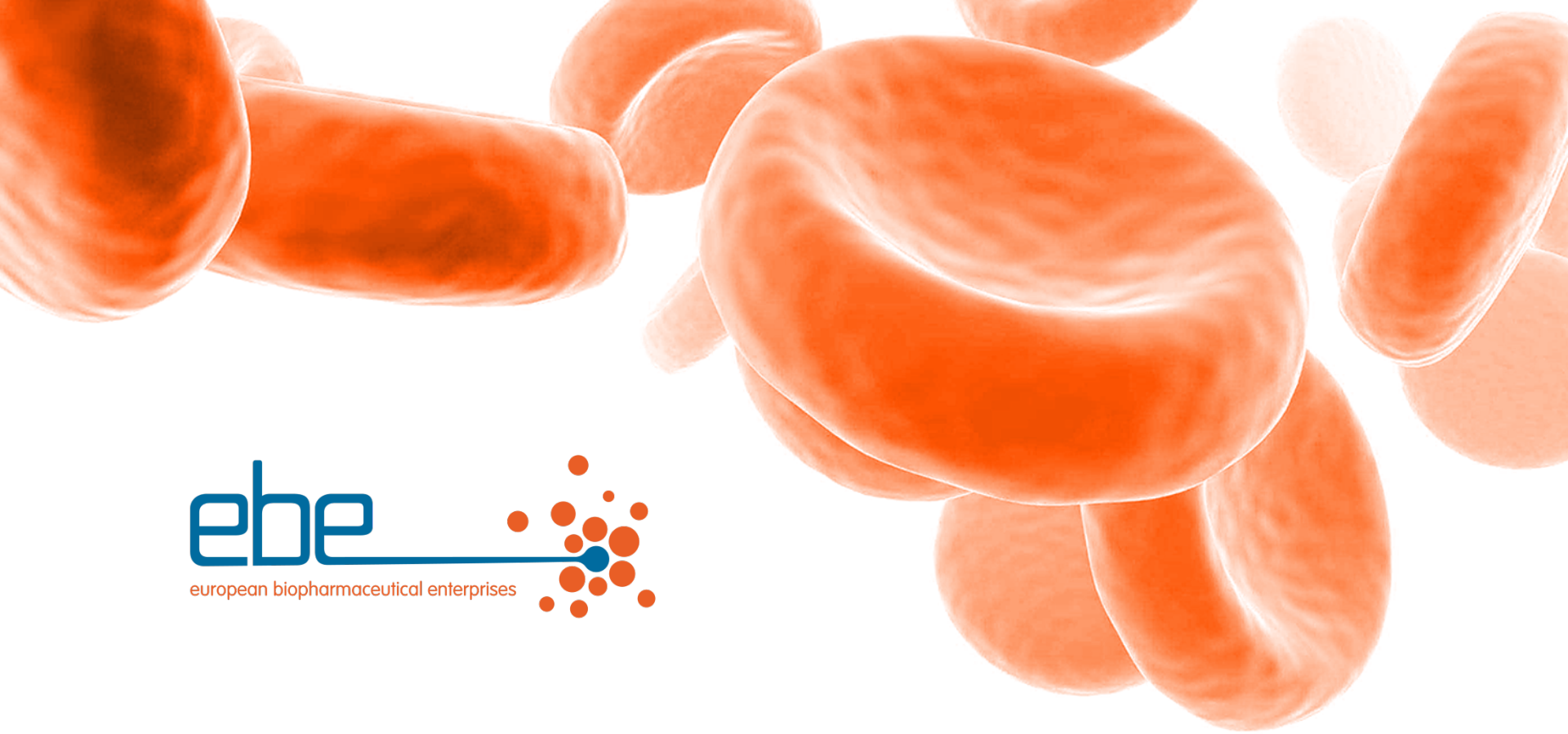

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A Risk-Based Approach to Setting Sterile Filtration Bioburden Limits

EBE Biomanufacturing Working Group Industry Consortium

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- The content of this slide deck is accurate to the best of the presenter's knowledge at the time of production.

Problem and objective statement

- No clear origin to the rationale behind the EMA* recommended bioburden limit before sterile filtration of not more than (NMT) 10 colony-forming units (CFU) / 100 ml. The limit has been taken from the pharmacopoeial specification for 'water for injection to produce bulk' and lacks scientific basis when applied to final drug products. According to the 1996/2016 guideline less than 100 ml sampling volume acceptable, if justified. But no guidance on how to "justify" given.
- EBE BWG position paper and follow-on paper provide a strategy and scientific methodology for **justifying alternate bioburden test limits / smaller test volumes as well as a case-by-case risk assessment approach for bioburden exceeding a predefined limit.**

* Draft Guideline of EMA on the sterilization of the medicinal product, active substance, excipient and primary container, 2016

Draft Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials, 2016

EMA (1996): CPMP Notes for Guidance on Manufacture of Finished Dosage Form.


EBE Position Paper

ebe

A Risk-Based Approach to Setting Sterile Filtration Bioburden Limits

12 October 2016 Version 1

Executive Summary

There is no clear origin to the rationale behind the EMA recommended bioburden limit before sterile filtration of not more than (NMT) 10 colony-forming units (CFU) /100 ml (EMA Guidelines of 1996, 2012 and Draft EMA Guideline 2016). The limit has been taken from the pharmacopoeial specification for 'water for injection to produce bulk', but has no scientific basis when applied to drug products. Instead, a "Risk-based Approach" to bioburden control is proposed, which justifies alternative bioburden specifications that take the product manufacturing process into consideration, in contrast to defaulting to the historical 10 CFU/100 ml limit.

This risk-based approach considers the two main risks at the sterile filtration step of drug product manufacture: (a) variability of the microbiological bioburden method and, (b) microbial breakthrough during sterile filtration. In the approach described in this Position Paper, these risks have been assessed for their interaction by means of statistics, and possible risk-mitigating measures are described.

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http://www.ebe-biopharma.eu/documents/86/61/EBE-Position-Paper-quot-A-Risk-based-Approach-to-Setting-Sterile-Filtration-Bioburden-Limits-quot

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The Risks

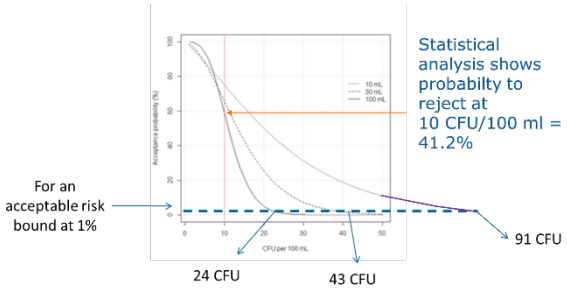
Three major risks identified:

- **Risk 1: Bioburden test method insensitivity (pre-sterile filtration risk due to method)**
  - Risk of false negative or passing a batch with unacceptable bioburden
- **Risk 2: Process-related risks and microbial breach across sterile filter (post-sterile filtration risk due to process)**
  - Risk of ≥ 1 CFU in filtered solution
- **Risk 3: by-products of bioburden**
  - microbial-derived components e.g. proteases, endotoxins and exotoxins

The Risk-based Approach regarding the sterile filtration process (Risks #1 and #2)

Step 1: Acceptable risk associated with bioburden test

- Negative binomial (NB) distribution to model bioburden distribution
- Establish test such that probability of passing batch with bioburden is bounded by a lower risk (e.g. 1% probability of false negative)
- Requires higher acceptable bioburden concentrations for detection



Step 2: Acceptable risk of ≥1 CFU in sterile filtered solution

- Depends on various process design parameters (demonstrated microbial retention, filter area, batch (filtration) volume, number of filters used in series or use of a bioburden reducing pre-filter)
- Set acceptable risk (risk bound) and couple with method insensitivity (pre-filtration) risk to determine overall combined risk

Step 3: Combined Risk Table → limit filtered batch size

Risk Bound		Pre-filtration Test Scheme		Maximum Bioburden[3] D_0	Maximum Batch Size
Pre-filtration[1]	Post Filtration[2]	Sample Volume V (mL)	Acceptance Limit AL (CFU/V)	(CFU/100 mL)	(L)
5%	10^{-4}	10	1	63	424
		30	3	32	826
		100	10	20	1355
	10^{-5}	10	1	63	42
		30	3	32	82
		100	10	20	135
1%	10^{-4}	10	1	91	297
		30	3	43	620
		100	10	24	1106
	10^{-5}	10	1	91	29
		30	3	43	62
		100	10	24	110
0.10%	10^{-4}	10	1	128	210
		30	3	58	465
		100	10	30	897
	10^{-5}	10	1	128	21
		30	3	58	46
		100	10	30	89

[1]Pre-filtration risk = Probability to pass a batch with a bioburden exceeding the maximum level D_0
[2]Post filtration risk = Probability to have ≥ 1 CFU in the final filtered solution
[3]Maximum bioburden D_0 = Maximum acceptable level of bioburden in the unfiltered solution

Example: Post-filtration risk of 10^{-4} = risk of microbial breach in 1 in 10,000 batches. For facility manufacturing 100 batches per year, one batch would have 1 CFU (in one vial) every 100 years.

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The holistic view: further mitigation of process-related risks in addition to limiting filtered batch size

- Use of closed systems, aseptic connections, and disposable processing components
- Defined hold times qualified by microbial and chemical stability
- Multiple bioburden reduction filtrations
- Filter integrity testing
- Selection of sterile filters with demonstrated microbial retention greater than 10^7 CFU/cm^2
- Larger filter surface area to volume ratio typically used in early development products
- Validated integrity of container closure system
- Qualified operators and validated interventions with media fills
- Validated CIP/SIP procedures
- Monitoring of facility capability (historical bioburden data), trending and setting of an internal limit

Bioburden Control Strategy

- Given the typically low (zero) pre-filtration bioburden, an **alert limit** should be defined rather than a fixed limit or acceptance criterion such as 10 CFU/100 ml. Trends towards more frequently observed bioburden excursions from historical data would trigger search for root cause → the facility is in a controlled state

Table: Pre-sterile filtration bioburden data taken over an extended period of time for 3 products

Product	Analyzed batches	Values without finding	Values with bioburden, but < 10 CFU/100ml	Values with bioburden > 10 CFU/100 ml	Quantile	Calculated level of control*	Excluded records
Product 1	516	511	6 (5x1, 1x2)	0	99% (neg. bin.**)	≤2 (neg. bin.**)	0
Product 2	318	313	5 (5x1)	0	99% (Poisson)	≤1 (Poisson)	0
Product 3	75	69	6 (3x1, 1x2, 1x4, 1x5)	1	99% (neg. bin.**)	≤ 4 (neg. bin.**)	1

*Specification applied: 10 CFU/100ml

**negative binomial

Case-by-Case assessment of contamination by-products (risk #3)

What are the risks of bioburden excursions ?

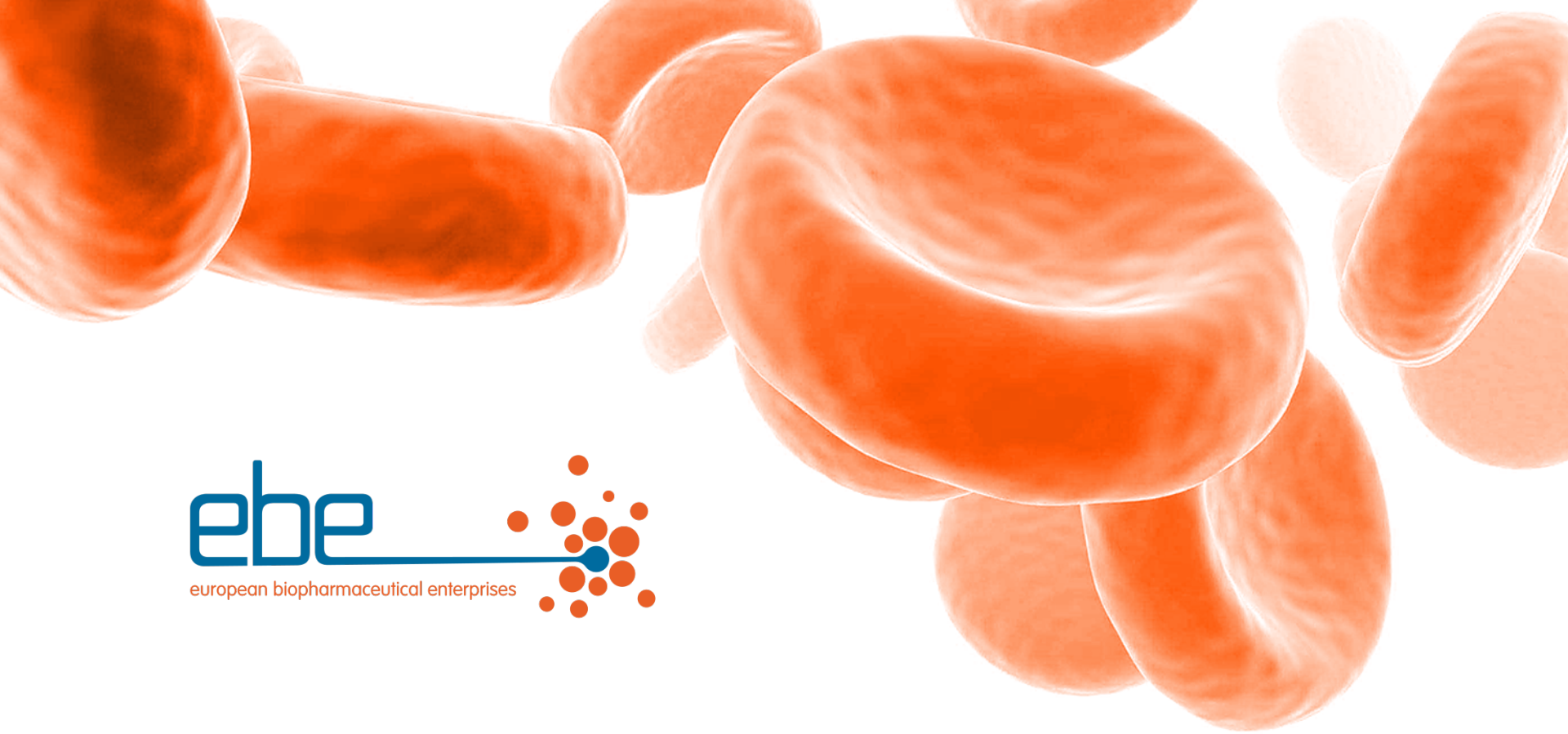
- Microorganisms might degrade or modify the product (e. g. by extracellular enzymes). Degraded or modified products might lose function or cause a safety issue. – however product integrity and biochemical purity are usually checked in the final drug product. There remains the risk that protein degradation may occur during storage.
- Microorganisms (gram-negative) release endotoxins (lipopolysaccharide = LPS), which might contaminate the drug solution and cause a safety issue – however endotoxins can be detected and quantified using the Bacterial Endotoxins Test (BET / LAL-assay). Defined endotoxin limits exist. For certain formulations endotoxins might be masked requiring further measures. Demasking strategies and monocyte activation test [Ph.Eur.] as possible alternative available.
- Microorganisms release other cellular components like exotoxins, DNA, flagella and/or lipopeptides / lipoproteins, which might contaminate the drug solution and cause a safety issue. - but potential load of lipopeptides / lipoproteins and exotoxins can be calculated (total bacteria mass → extracellular proteins or total microbial protein content → potential exotoxin as well as potential lipopeptide / lipoprotein load). This includes gram-positive and gram-negative bacteria[1].

[1] F. von Wintzingerode, American Pharmaceutical Review, April 2017, pages 10 - 19

Conclusions/ Position Statement

- Risk-based approaches are consistent with ICH guidance
- Risks involving bioburden test and process can be quantitated and acceptable risk tolerance be utilized
  - Analogies can be made to AQL testing and acceptable sterility assurance level (SAL)
- By-products due to bioburden exceeding predefined limits can also be calculated and the risk assessed in a case-by-case risk assessment (CCRA or CCAB) and documented in the QMS

Position: Smaller bioburden test volumes (e.g., 10 ml) do not increase risk significantly for typical processes. Methodology can be used to justify alternate limits to 10 CFU/100 ml. Batches exceeding pre-defined bioburden limits pre-sterile filtration may be released following a systematic CCRA/CCAB (if assessment favourable).



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A Risk-Based Approach to ID Sampling of Biologics Drug Substances

EBE Biomanufacturing Working Group Industry Consortium

The Problem

100% Containerwise ID Sampling & Testing of Incoming DS

- EBE survey indicates that 100% containterwise testing (ie thawing, opening, sampling every bulk DS unit) has been cited by inspectors during on-site inpections as a regulatory requirement and that travel/satellite samples have not been accepted.
- It appears that a new interpretation is being applied by EU inspectors to cGMP ID testing of incoming bulk DS shipments of biopharm products
  - *QUESTION: What has caused a change in this EU inspectional interpretation on the regulatory side?*
- **EBE Member Company Survey:** Biotech industry has applied various interpretations of the sampling required for cGMP ID testing of bulk DS shipments:
  1. No ID testing of the received bulk DS batch (primarily internal site to site DS shipment)
  2. Testing the received bulk DS shipment for representative DS satellite sample(s) prepared during DS fill at DS manufacturing site
  3. 100 % Containerwise sampling individual containers of the received bulk DS shipment at DP site

Current Regulations for cGMP ID Testing

Eudralex Vol 4 GMP Annex 8: Sampling of Starting Material and Packaging Materials

It is permissible to sample only a proportion of the containers where a validated procedure has been established to ensure that no single container of starting material has been incorrectly labelled…. Under such a system, it is possible that a validated procedure exempting identity testing of each incoming container of starting material could be accepted…"

FDA "Questions and Answers on Current Good Manufacturing Practices, Good Guidance Practices, Level 2 Guidance - Control of Components and Drug Product Containers and Closures"

"These regulations require representative samples of each shipment of each lot of active and inactive component (or raw materials) to be tested to confirm the identity of the component as labeled prior to release for use in drug product manufacturing …The CGMP regulations do not specify the number of containers to be sampled from each received shipment. … The CGMPs permit each drug product manufacturer to make its own decision as to the number of containers to sample, as long as the sampling plan is scientifically sound, leads to representative samples, and complies with the principles established at 21 CFR 211.84(b)."

Both regulations allow less than 100% sampling (ie each container), based on a scientifically sound, validated procedure

Unique Risks

100% sampling of containers of biological products introduces two significant risks to product quality:

- Thawing and re-freezing every bulk DS container introduces more physical stress on the product that can increase the risk of aggregation in the DS solution
- Sampling every aseptically-filled bulk DS container increases the risk of microbial contamination from the sampling operation

In addition, 100% sampling introduces logistical complications to manufacturing and testing:

- Large numbers of low volume DS containers must be sampled, processed and tested for ID, increasing time and costs
- To assure stability, DS units must be re-frozen and held pending ID test results
  OR
  Aseptic processing must proceed before results of containerwise ID testing are available to avoid lengthy hold of thawed DS containers prior to sterilizing filtration

EBE Recommendations for cGMP ID testing of Biologics

(Continue to) utilize a scientifically sound, validated, risk-based approach for ID verification without mandating 100% container-wise sampling and testing of thawed Drug Substances upon receipt by Drug Product manufacture.

- Representative samples may be collected at the time of DS fill ('satellite' samples) and can be acceptable where procedural and quality requirements are defined.
- Suitability of the DS manufacturer quality system must be verified and continued compliance to defined quality system procedures is ensured.
- A program for satellite samples must be procedurally defined at both DS and DP sites. The procedures must be suitably validated and monitored.
- Sample(s) collected during DS containers fill must be representative of the entire DS batch
- Satellite samples must be shipped with DS batch containers as a unit (i.e. not as pre-shipment samples).
- Appropriate controls must be in place for
  - Labeling, identification and reconciliation
  - Secure shipping and transport
  - Appropriate monitoring of transport and documented chain of custody
  - Receipt of shipment and verification at DP manufacturing site

Team Composition

- Team Bioburden Risk-based Approach
  - Karoline Bechtold-Peters, Novartis
  - David Roesti, Novartis
  - Friedrich von Wintzingerode; Roche
  - Christian Matz, Roche
  - Benoit Ramond, Sanofi
  - Andrew Lennard, Amgen
  - Jeanne Mateffy, Amgen
  - Harry Yang, Medimmune - AstraZeneca
  - Steven Chang, Medimmune - AstraZeneca
  - Melvyn Perry, Pfizer
  - Donald C. Singer, GSK
  - Julian Kay, GSK
  - Paola Barzi, MerckGroup
  - Frederik Intelmann, Boehringer Ingelheim
  - Liesbeth Voeten, Janssen
  - Anette Yan Marcussen, Novonordisk
  - Lilly is a consortium member of EBE
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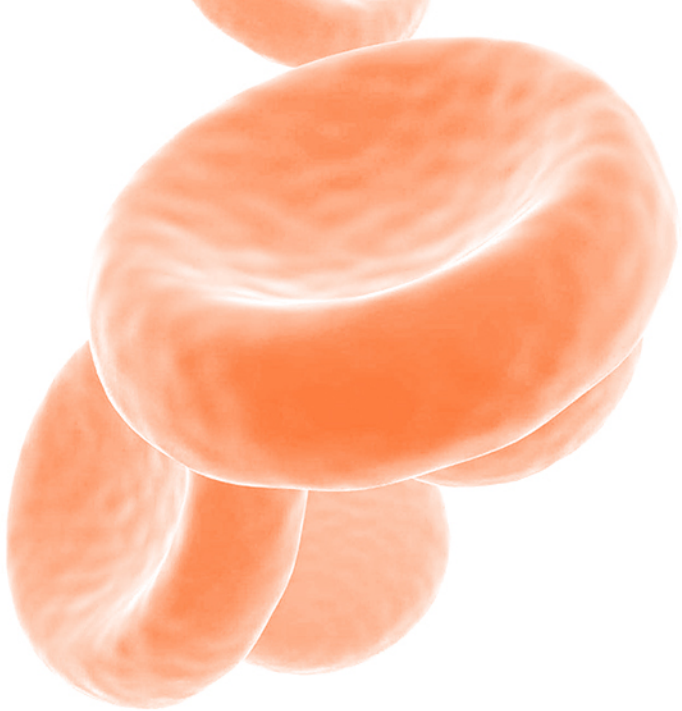
  - Aldick, Thomas, Roche
  - Andrea Calenne, Biogen
  - Huub Strouken, Roche
  - Kaat de Moor, Synthon
  - Philippe Dupont, Biophytis
  - Saroj Ramdas, GSK
  - Jennifer Walraven; GSK
  - Wendy Zwolenski-Lambert, Novartis
  - Nadine Ritter, consulting



Back-up Slides

Systematics of a Case-by-case Risk Assessment of Bioburden – example calculations

- Molecular identification of bacteria by 16S rDNA sequencing
- Literature search for exotoxins, MALP-2 like lipopeptides/proteins, etc.
- Calculation of hypothetical toxic load based on
  - Cell number (CFU) per mL
  - Cell volume, cell mass and hence the total microbial protein content
    - Exotoxins (as % of total microbial protein content)
    - Lipopeptides/proteins (as % of total microbial protein content)
    - Other by-products such as flagellin, DNA, cell wall polysaccharides,....
  - Drug dose per day
- Assessment of risk potential
  - Protein exotoxins: The calculated dose of toxin is noncritical if it is less than the TDLo* of botulinum toxin A (1.2 pg/kg body weight).
  - Lipopeptides/proteins: The calculated dose of toxin is non-critical if it is less than the TDLo* of lipopolysaccharide (LPS = endotoxin) (4 ng/kg body weight).
  - .....

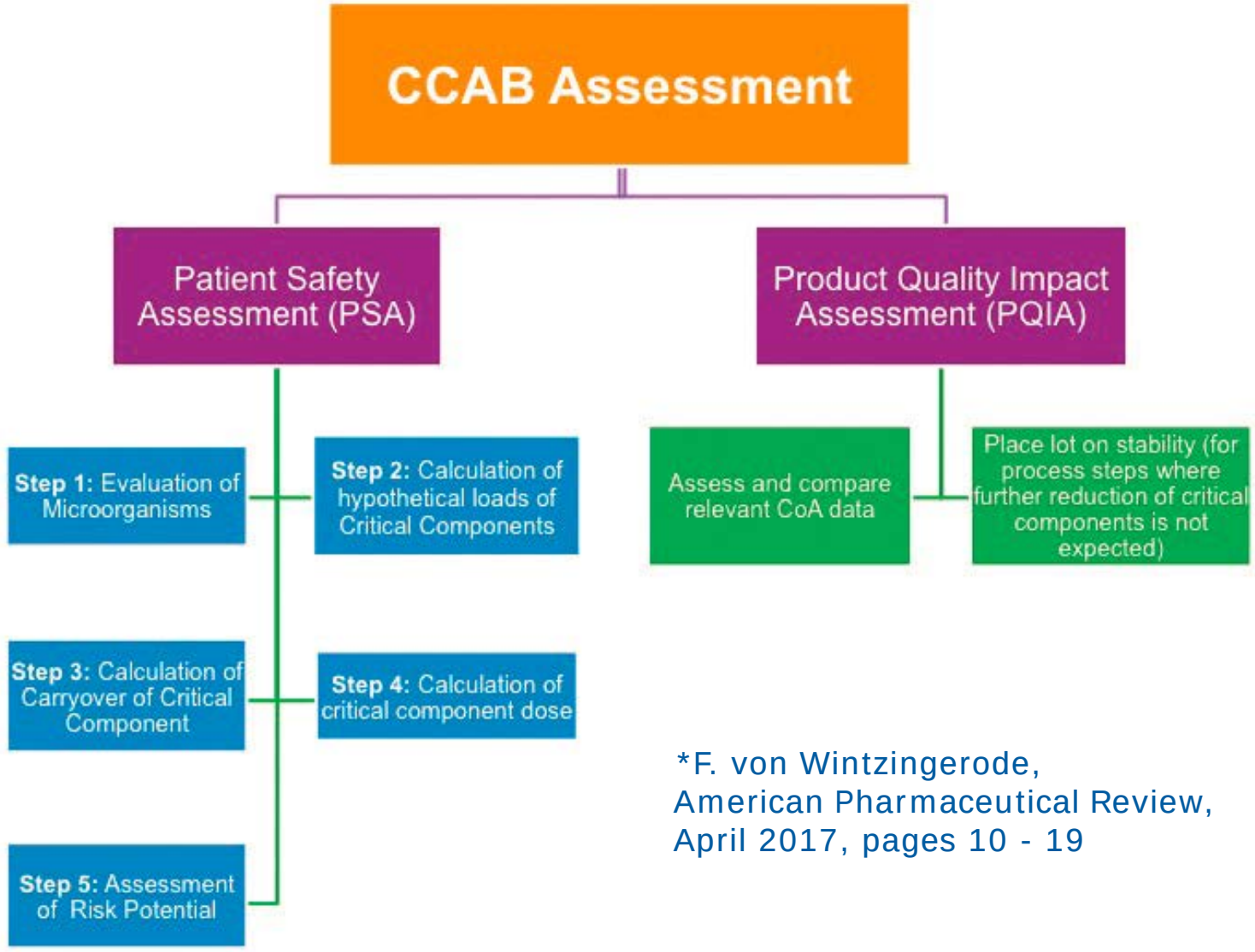
Example calculation

Calculation of potential contamination per kg body weight and day [ng/kg]

Calculated value	Result	units	Detailed information/calculation
Contamination (Bioburden)	53	cfu / ml	
Volume of single cell	3.93	µm³	(assumption: rod shape organism); V = (d/2)² x π x l; d = 1.0 µm, l = 5.0 µm)
Moist mass of single cell	3.93	pg	(density = 1 pg/µm³)
Total moist mass in storage vessel	208.3	pg / ml	(3.93 pg x contamination 53 cfu / ml)
Total dry mass	62.5	pg / ml	(15 - 30% --> worst case 30% of moist mass)
Total protein content	34.4	pg / ml	(55% of dry mass)
Mass of potential toxin (worst case)	34.4	pg / ml	Worst case assumption → 100% of dry mass are toxins Extracellular proteins
Maximum human dose	98.8	ml	
Potential protein exotoxin contamination per dose	3399	pg	(98.8 ml x 34.4 pg/ml)
Potential protein exotoxin contamination per kg body weight	136	pg / kg	(body weight child 25 kg)
TDL_o Botulinum Toxin A	1.2	pg / kg	RTECS
Conclusion			
Potential exotoxin contamination below the TDL_o of Botulinum Toxin A?	☒ yes ☐ no		

*Toxic dose low. Based on the RTECS Guideline (US Dept. of Health and Human Services), the lowest dose of a substance which, whatever the dosage form and over an indeterminate time period, causes a documented toxic effect in humans

Key aspects of CCRA/CCAB approach*



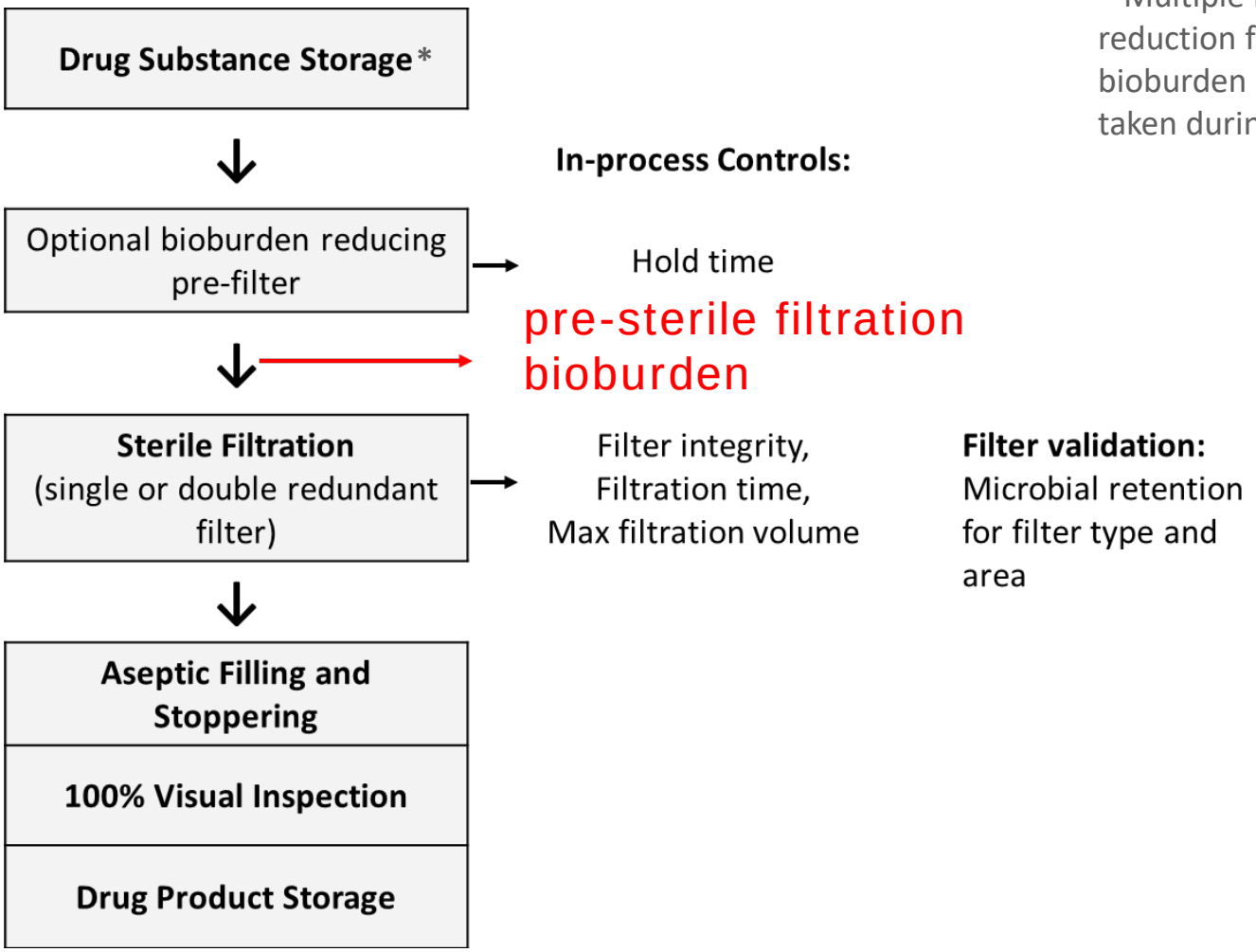
*F. von Wintzingerode, American Pharmaceutical Review, April 2017, pages 10 - 19

Endotoxins

- Endotoxins can be detected and quantified using the Bacterial Endotoxins Test. Defined endotoxin limits exist. Therefore release of endotoxins as a consequence of exceeding bioburden limits is not a critical issue.
- However a couple of years ago the masking of endotoxins also referred to as a low endotoxin recovery effect (LER effect) was detected by one company and communicated to FDA.
- Low endotoxin recovery (LER) can be defined as the failure to detect known amount of endotoxin in a biological product using the compendial bacterial endotoxin test (Limulus Amoebocyte Lysate, LAL) despite the fact that the positive controls show no evidence of inhibition and is typically characterized by a decline in the measurable endotoxin concentration over time (such as during sample storage).
- Based on current knowledge the biological products with the highest risk of inducing LER are the ones which contain polysorbate and high molecular weight proteins or chelating agents.
- Different strategies exist in the industry to deal with LER such as further tightening of the endotoxin burden controls, use of demasking procedures or introduction of the rabbit pyrogen test as release test. Furthermore the Monocyte Activation Test (MAT) is a possible alternative test (Ph.Eur.) and Naturally Occurring Endoxtoxins may be used instead of LPS (NOE, Reference Standard in development by USP)[1]

[1] However, FDA does not accept NOEs as appropriate LER mitigation strategy

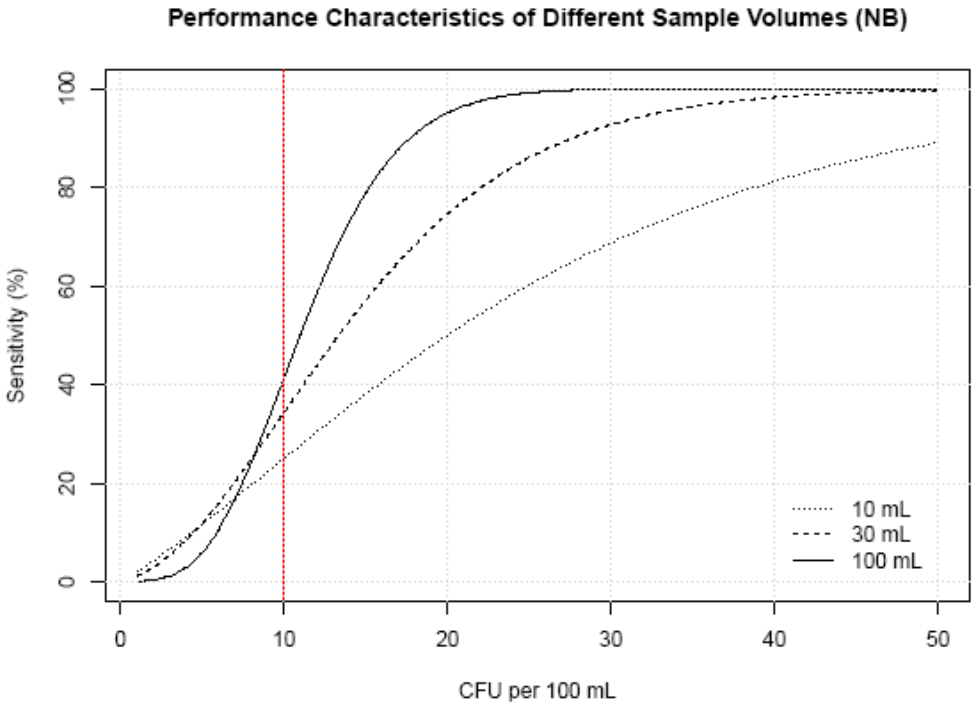
Process Flow Diagram for Manufacture of a Sterile Liquid Drug Product



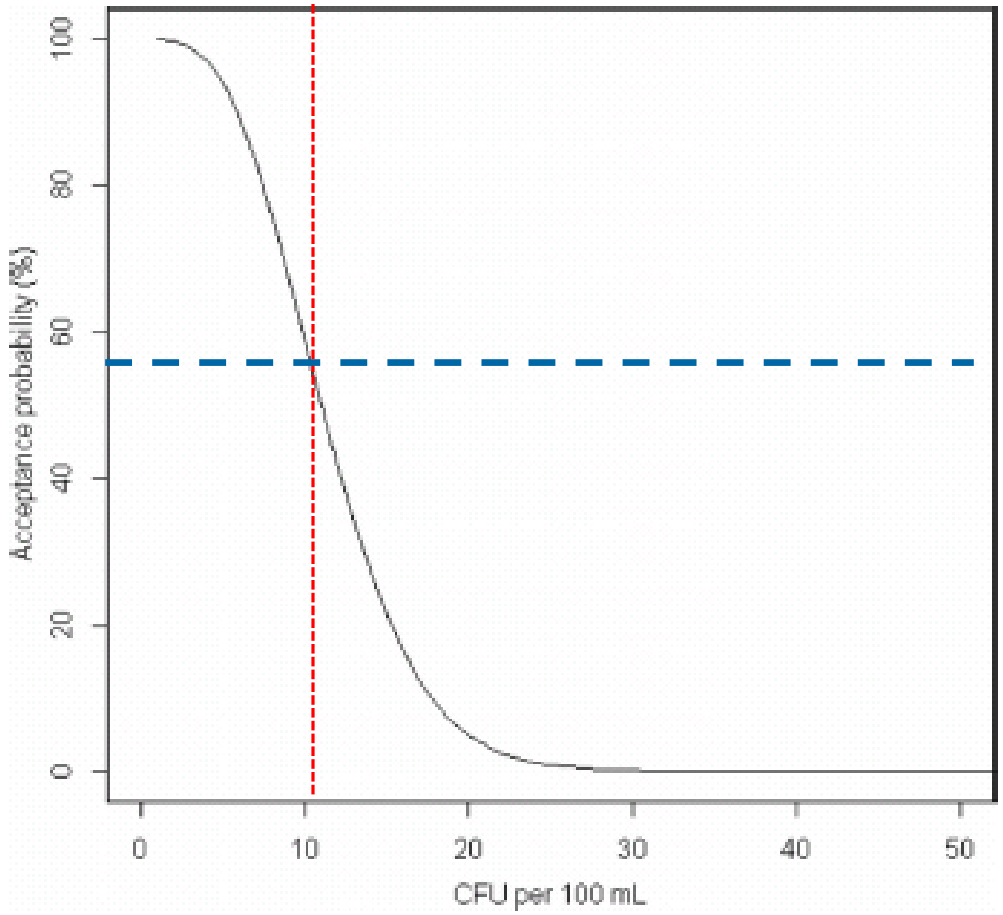
* Multiple bioburden reduction filtrations and bioburden samples typically taken during DS manufacture

Modeling bioburden distribution in solution to quantitate method insensitivity (pre-filtration risk)

- Negative binomial (NB) distribution used to model bioburden distribution
- NB model allows for bacterial clumping with better representation of "microbiological environment" compared to uniform (Poisson) distribution
- Statistical analysis shows sensitivity (probability) to detect 10 CFU/100 mL = 41.2%
- At same 41.2% sensitivity level, corresponding bioburden level for different sample test volumes can be calculated



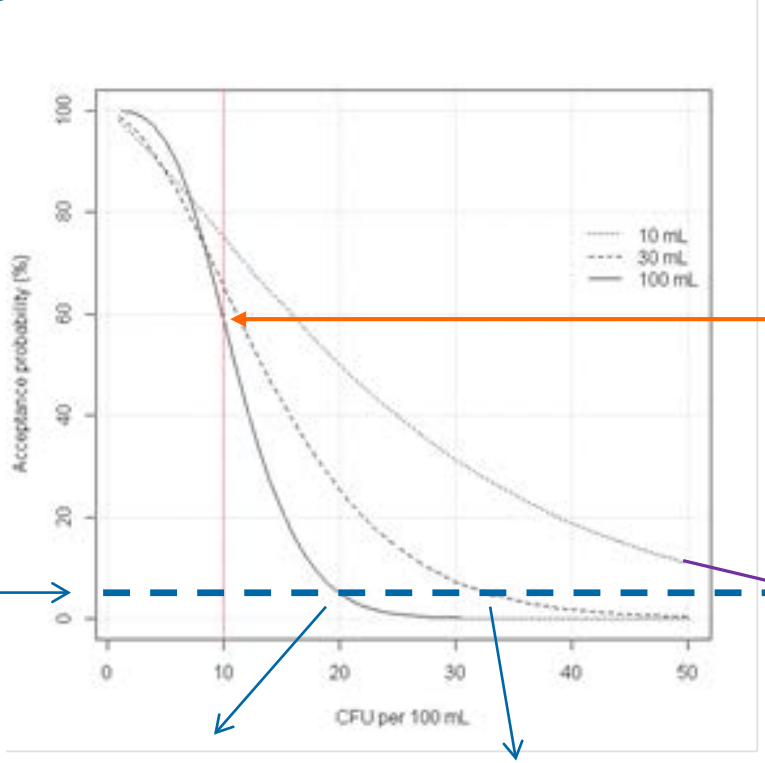
Limited method sensitivity means risk of passing batches with bioburden



Actual Bioburden (CFU/100 ml)	Probability (to pass bioburden test)
9	66.6%
10	58.8%
11	50.0%

Step 1: Control risk associated with bioburden test

- Negative binomial (NB) distribution used to model bioburden distribution
- Establish test such that probability of passing batch with bioburden is bounded by a lower risk, i.e. 5% probability of false negative
- Requires higher acceptable bioburden concentrations for detection



Statistical analysis shows probability to reject at 10 CFU/100 mL = 41.2%

For an acceptable risk bound at e.g. 5%

20 CFU

32 CFU

63 CFU

Step 2: Assess Risk of ≥1 CFU in sterile filtered solution

- Depends on various process design parameters
  - Demonstrated microbial retention (filter validation studies), i.e. 10^7 CFU/ cm^2 or greater for particular filter type
  - Filter area
  - Batch (filtration) volume
  - Number of filters used in series or use of a bioburden reducing pre-filter
- Quantitate risk and couple with method insensitivity (pre-filtration) risk to determine overall combined risk

Step 3: Combined Risk Table

(example for single filter, 10^7 CFU/cm^2 retention capability, and 1000 cm^2 area)

Risk Bound		Pre-filtration Test Scheme		Maximum Bioburden[3] D_0 (CFU/100 mL)	Maximum Batch Size (L)
Pre-filtration[1]	Post Filtration[2]	Sample Volume V (mL)	Acceptance Limit AL (CFU/V)		
5%	10^{-4}	10	1	63	424
		30	3	32	826
		100	10	20	1355
	10^{-5}	10	1	63	42
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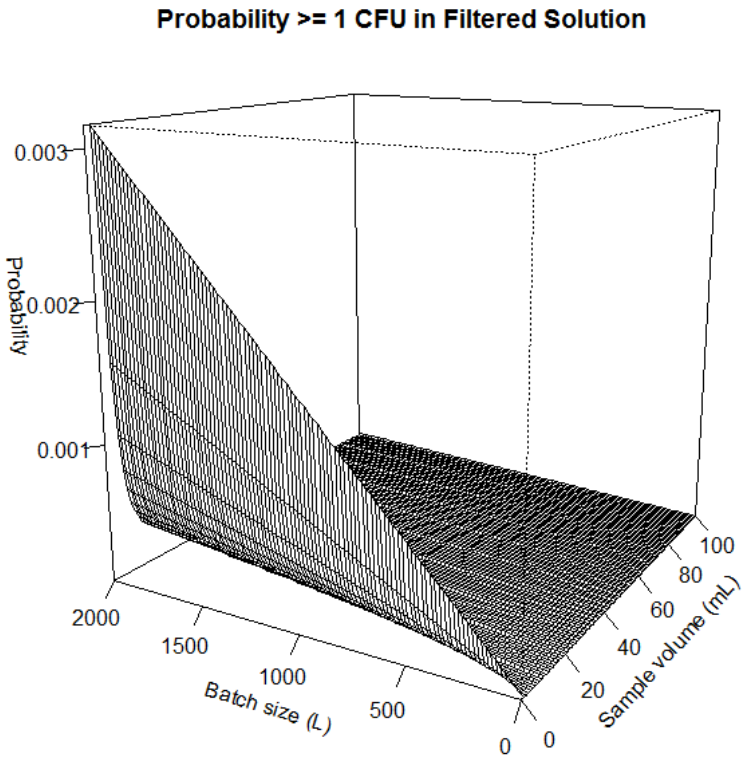
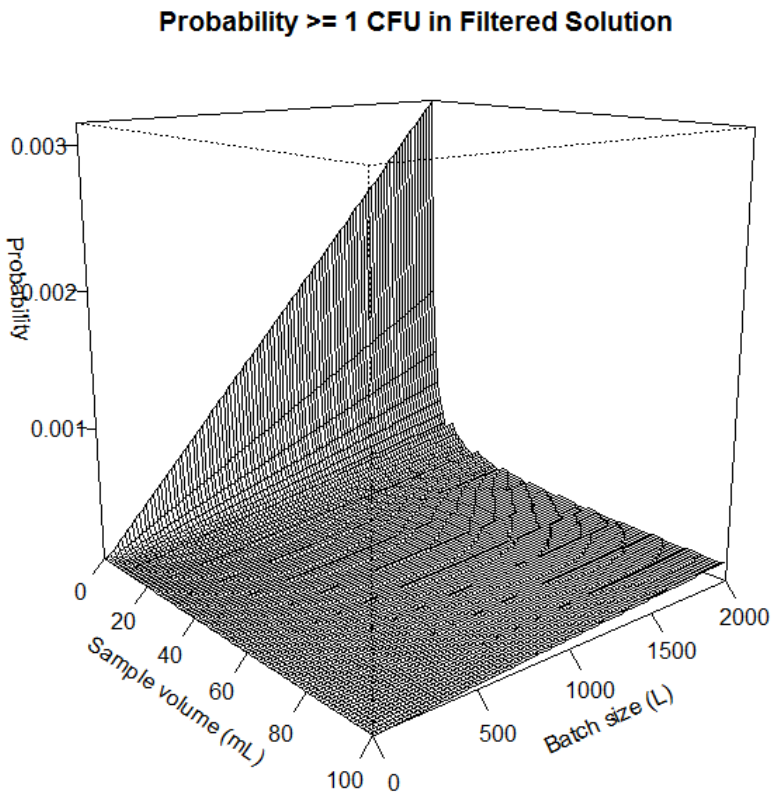
		30	3	58	465
		100	10	30	897
	10^{-5}	10	1	128	21
		30	3	58	46
		100	10	30	89

[1]Pre-filtration risk = Probability to pass a batch with a bioburden exceeding the maximum level D_0
[2]Post filtration risk = Probability to have ≥ 1 CFU in the final filtered solution
[3]Maximum bioburden D_0 = Maximum acceptable level of bioburden in the unfiltered solution

- Post-filtration risk of 10^{-4} = risk of microbial breach in 1 in 10,000 batches. For facility manufacturing 100 batches per year, one batch would have 1 CFU (in one vial) every 100 years.
- Retention of max bioburden D_0 well within typical process and filter retention capacities

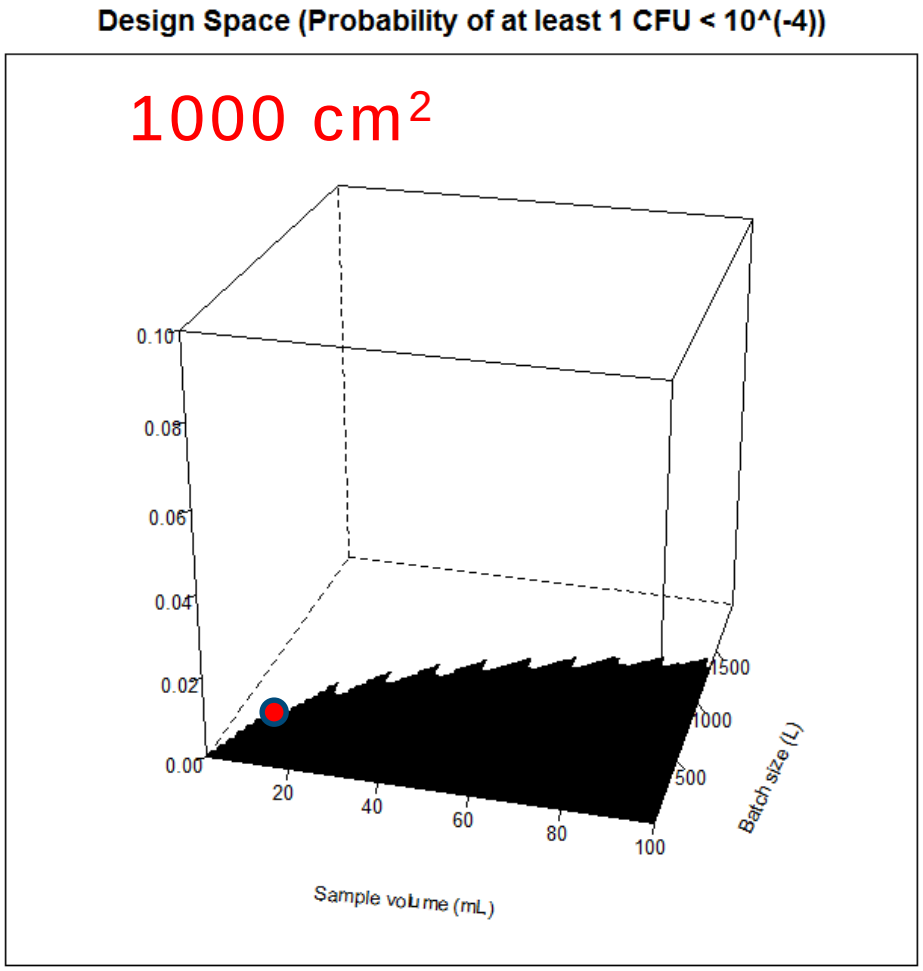
Corresponding risk contour plots
(Bound at 5% pre-filtration risk)





Probability is strong function of batch size at small sample volumes

Resulting design space within risk tolerance
(Bound at 5% pre-filtration risk, 10^{-4} post-filtration risk)

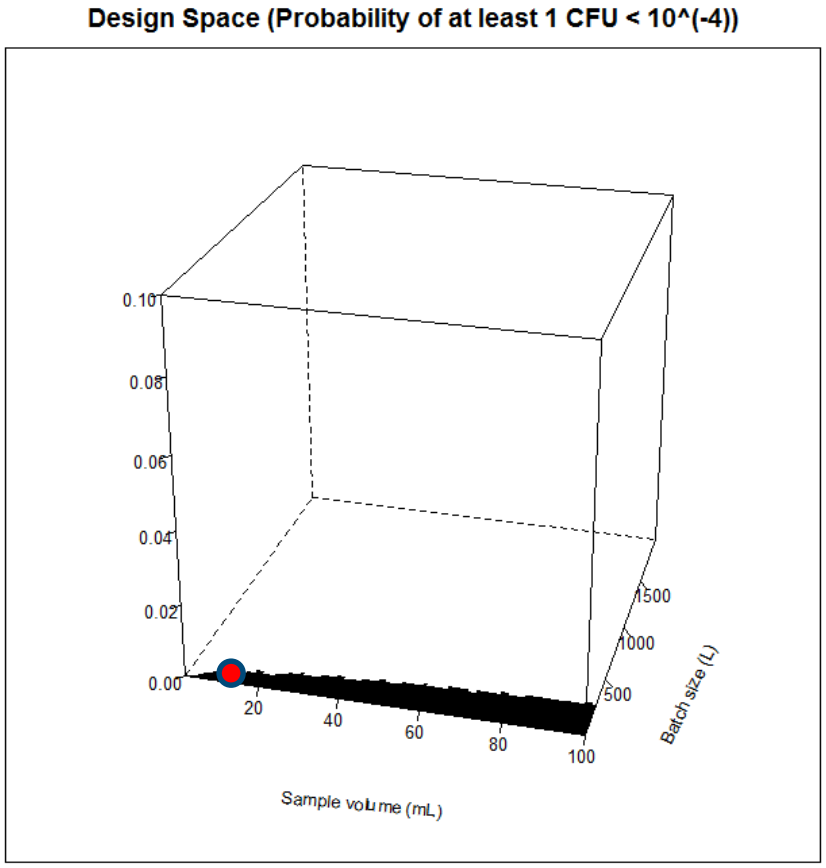


- Example:
  - Limit of 1 CFU/10 ml for max batch volume of 424 L would be within stated risk tolerance

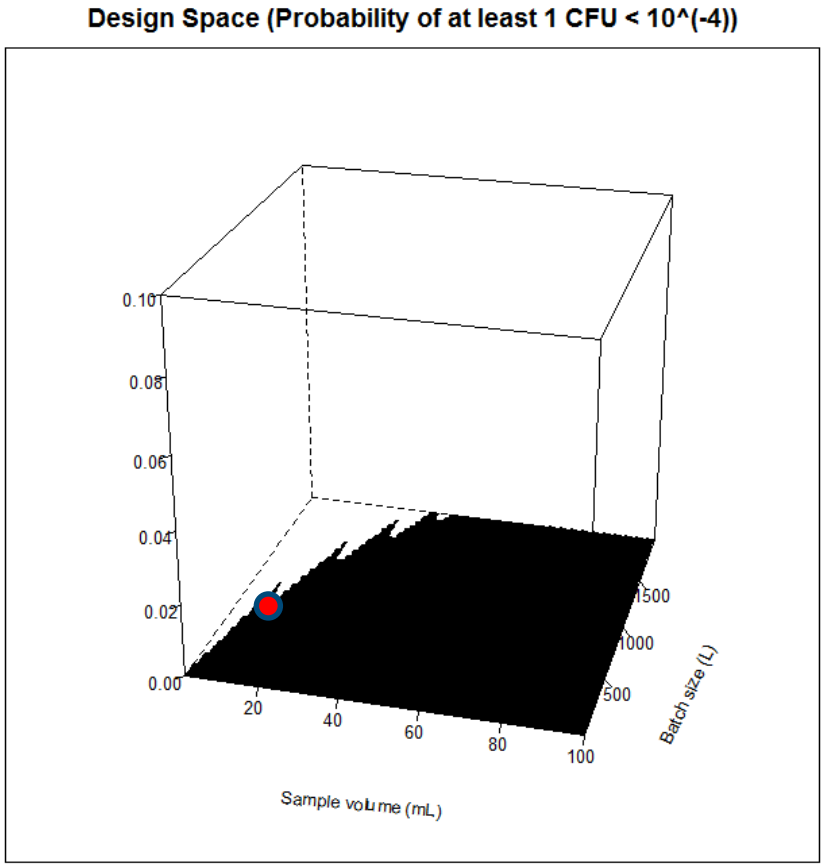
    (5% probability of false negative and breach in 1 in 10,000 batches)

Different design scenarios: Filter area
(Bound at 5% pre-filtration risk,10^{-4} post-filtration risk)

200 cm^2



2000 cm^2

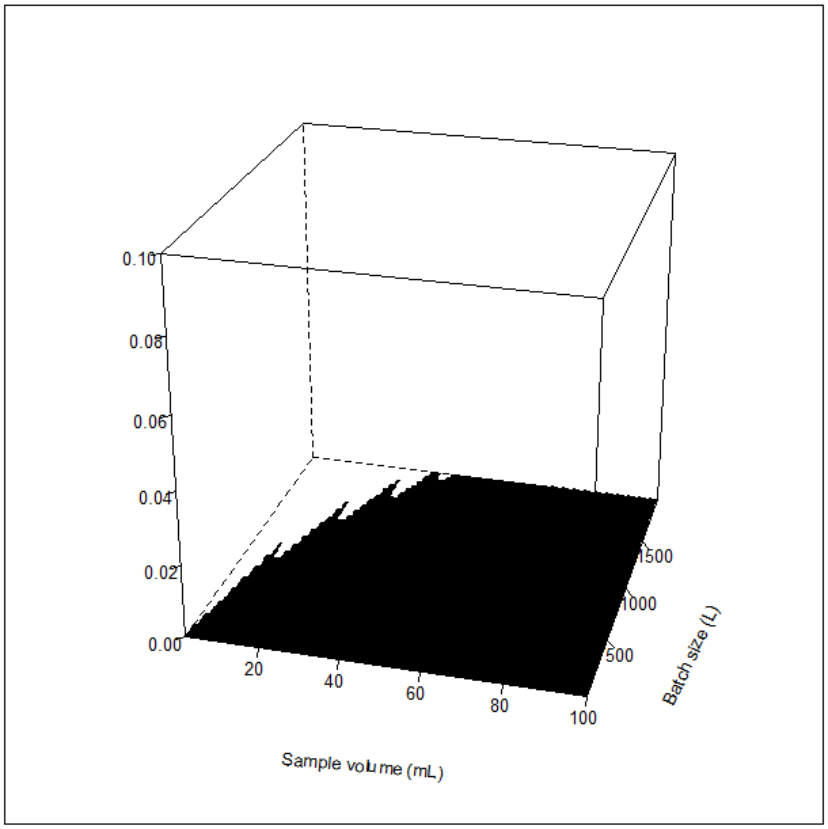


Here:

- Limit of 1 CFU/10 ml for max batch volume of 85 L (200 cm^2) or 849 L (2000 cm^2) would be within stated risk tolerance (5%/10^{-4})

Different risk tolerance scenarios: Process risk
(Bound at 5% pre-filtration risk, varying post-filtration risk)

10^{-4}



10^{-5}