

Simple Bioburden Monitoring for Contamination Control

Simple and fast Bioburden
Monitoring with the Sievers*
Rapid Bioburden Instrument

19th of June 2023
24. Swiss Cleanroom Community Event
Presented by: Dr. Eugen Federherr

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RAPID BIOBURDEN MONITORING

Bioburden is the total number of microorganisms associated with a specific item such as personnel, manufacturing environments (air and surfaces), equipment, product packaging, raw materials (including water), in-process materials, or finished products.

FACILITY EQUIPMENT AND PROCESS

The use of appropriate technologies (e.g. Restricted Access Barriers Systems (RABS), isolators, robotic systems, **rapid/alternative methods** and continuous monitoring systems) **should be considered to increase the protection of the product from potential extraneous sources of endotoxin/pyrogen, particulate and microbial contamination** such as personnel, materials and the surrounding environment, and assist in the rapid detection of potential contaminants in the environment and the product.

ENVIRONMENTAL AND PERSONNEL MONITORING

The adoption of suitable alternative monitoring systems such as rapid methods should be considered by manufacturers in order to expedite the detection of microbiological contamination issues and to reduce the risk to product.

QUALITY CONTROL

For products with short shelf life, the environmental data for the time of manufacture may not be available; in these cases, the compliance should include a review of the most recent available data. Manufacturers of these products should consider the use of rapid/alternative methods.

TRADITIONAL BIOBURDEN TESTING METHOD

MEMBRANE FILTRATION PLATE COUNT

1



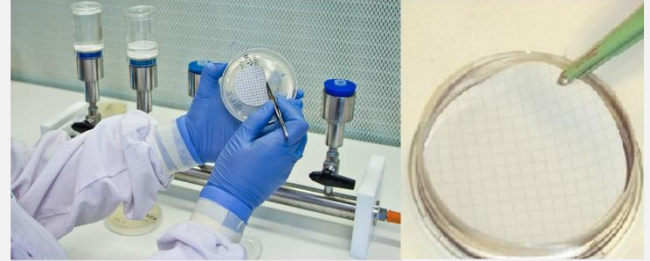
Collect Sample in Plant

2



Transport sample to Lab and filter water through Membrane Filter

3



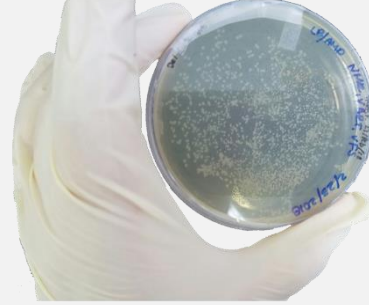
Place Membrane Filter on appropriate Nutrient Medium

4



Incubate for 2-5 days or longer

5



Manually count viable cell colonies that have grown on the plate

Why wait
3-5 days
or more
for results?



PROBLEM

Bioburden results are not available in time for effective process monitoring and product release.



Current growth-based methods provide acceptable accuracy since 1905

--- however ---

We wait 2 – 5+ days for results

Retrospective versus actionable process controls



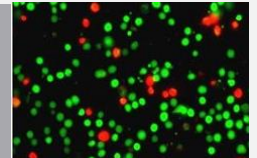
SOLUTION:

Sievers® Soleil Rapid Bioburden
Accurately count <10 cells in 100mL of sample

Bioburden results in <60 minutes reduce operating costs and improve risk management

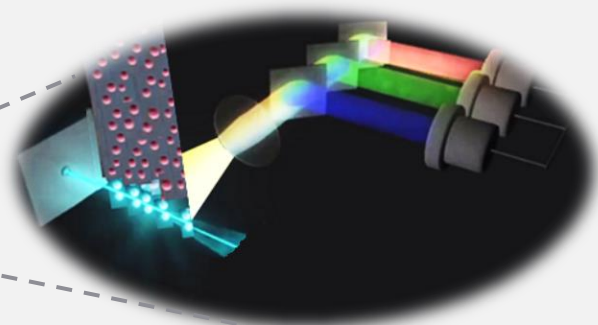
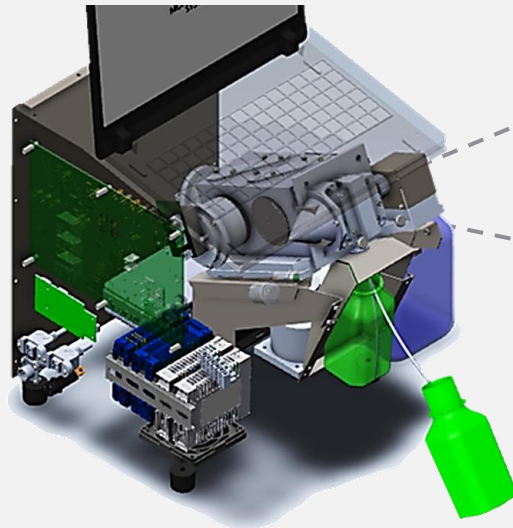


Viability stains in combination with ultra-sensitive, high throughput flow cytometer



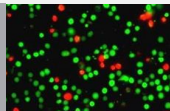
Sievers® Soleil Rapid Bioburden

Accurately count <10 cells in 100mL of sample in <60 minutes

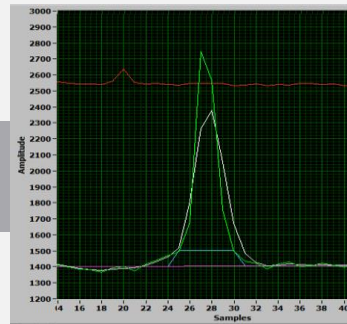


Ultra-sensitive high throughput flow cytometer

+ Viability stains



+ Spectral analysis



= Accurate quantification of viable organisms

RAPID BIOBURDEN IN <60 MINUTES

EASY TO USE IN LAB OR AT-LINE

01

Thermal
stabilization



02

Add
reagents



03

Incubate



04

Add
counterstain



05

Process on
Analyzer



First 100mL assay
< 60 min

Next assays
<30 minutes



SIEVERS RAPID BIOBURDEN APPLICATIONS

Sievers Rapid Bioburden systems can play a key role in routine bioburden monitoring for **cost savings while improving contamination control and manufacturing agility**, including:

- Pharmaceutical manufacturing water – critical control points
- Cleaning validation / hygiene monitoring
- Process water
- Raw materials
- At-line monitoring of drug substances and intermediate products
- Environmental monitoring of process areas (surface, air, etc.)



VERIFIED CORRELATION TO PLATE COUNTS

100mL SAMPLES

Mixed culture inoculated into purified water containing equal contributions of *B.cepacia*, *R.pickettii*, *S.maltophilia*, and *E.coli*

Sample Description	Target Count per 100 mL	Average Plate Count per 100 mL	Sievers® Rapid Bioburden count per 100 mL	Sievers® Rapid Bioburden % correlation to Plate	Average Plate Count (CFUs)	Average Sievers® Rapid Bioburden	STDEV Sievers® Rapid Bioburden	CV Sievers® Rapid Bioburden	Sievers® Rapid Bioburden % correlation to Plate
WFCC Neg Con - n1	0	0	0	100%	0	0	0	0%	100%
WFCC Neg Con - n2	0		0	100%					
WFCC Neg Con - n3	0		0	100%					
MixedCulture 0.05 - n1	5	6	5	83%	6	7	2	29%	117%
MixedCulture 0.05 - n2	5		7	117%					
MixedCulture 0.05 - n3	5		9	150%					
MixedCulture 0.1 - n1	10	12	13	108%	12	13	4	26%	111%
MixedCulture 0.1 - n2	10		17	142%					
MixedCulture 0.1 - n3	10		10	83%					
MixedCulture 0.5 - n1	50	66	75	114%	66	67	10	15%	101%
MixedCulture 0.5 - n2	50		56	85%					
MixedCulture 0.5 - n3	50		69	105%					
MixedCulture 1 - n1a	100	127	102	80%	127	117	16	14%	92%
MixedCulture 1 - n2a	100		116	91%					
MixedCulture 1 - n3a	100		134	106%					
MixedCulture 1 - n1b	100	113	141	125%	113	132	10	8%	117%
MixedCulture 1 - n2b	100		121	107%					
MixedCulture 1 - n3b	100		134	119%					
MixedCulture 10 - n1	1,000	1,067	1,264	118%	1,067	1,289	36	3%	121%
MixedCulture 10 - n2	1,000		1,330	125%					
MixedCulture 10 - n3	1,000		1,274	119%					
MixedCulture 100 - n1	10,000	11,000	12,188	111%	11,000	12,218	199	2%	111%
MixedCulture 100 - n2	10,000		12,036	109%					
MixedCulture 100 - n3	10,000		12,430	113%					

Results from one of the world's largest biotech companies



VBmicro - a suitable lab device for bioburden determination of water and buffers in less than 1hour

European Microbiology Conference 2021



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Information Source

Water: WFI Background

Date	Sample	Results with software adaption	
		Biotics/100ml	Abiotics/100ml
08.10.2020	WFI	0	92
08.10.2020	WFI	3	95
08.10.2020	WFI	0	81
08.10.2020	WFI	0	121
08.10.2020	WFI	1	117
08.10.2020	WFI hot 1	0	175
08.10.2020	WFI hot 2	0	163
20.10.2020	WFI	0	68
20.10.2020	WFI	0	23
01.02.2021	WFI hot	0	250
01.02.2021	WFI hot	0	238
01.02.2021	WFI hot	1	390
01.02.2021	WFI hot	0	223

→ WFI background: 0 – 3 biotics/100ml (action limit: 10 cfu/100ml)



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Comparison to standard plate count method

VBmicro vs. membrane filtration

Setup: Different dilutions of tap water (as contamination source) in sterile water

→ Parallel analysis with VBmicro* and filtration of same sample

Sample	cfu/ 100ml	Average [biotics/ 100ml]	Result 1 [biotics/ 100ml]	Result 2 [biotics/ 100ml]
0.0001% TW	4	2	1	3
0.0005% TW	10	8	6	10
0.001% TW	12	19	15	23
0.002% TW	28	32.5	29	36
0.005% TW	58	68.5	68	69

Correlation VBmicro vs. Plate count within 50-200%



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In Process Control: Buffer samples

- Potentially contaminated (*Paenibacillus*) buffer samples in downstream process of DS production
- Fast VBmicro analysis result (140x faster than plate count result)

Batch	VBmicro Results				Plate Count
	Run 1 [biotics/20ml]	Run 2 [biotics/20ml]	Run 3 [biotics/20ml]	Average [biotics/10ml]	QC result [cfu/10ml]
A	0	0	1	0.2	0
B	0	0	1	0.2	0
C	7	13	28	8	0
D	124	132	153	68.2	44
E	83	83	97	45.5	24
F	366	365	307	137	59
G	1	0	1	0.3	0
H	93	83	98	45.7	14
I	33	33	42	18	10
J	322	306	305	155.5	41
K	5	6	7	3	0
L	112	110	119	56.8	27
M	134	131	147	68.7	38
N	253	297	262	135.3	98



→ faster decision making possible for future process steps



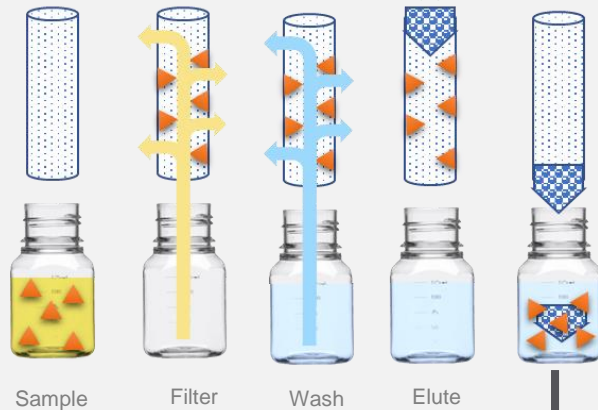
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Verified sample preparation method for complex samples including raw materials, drug substances, and products for “At-Line” or lab-based testing station

Result in < 60 minutes with ~ 15 minutes hands-on time for 100mL sample

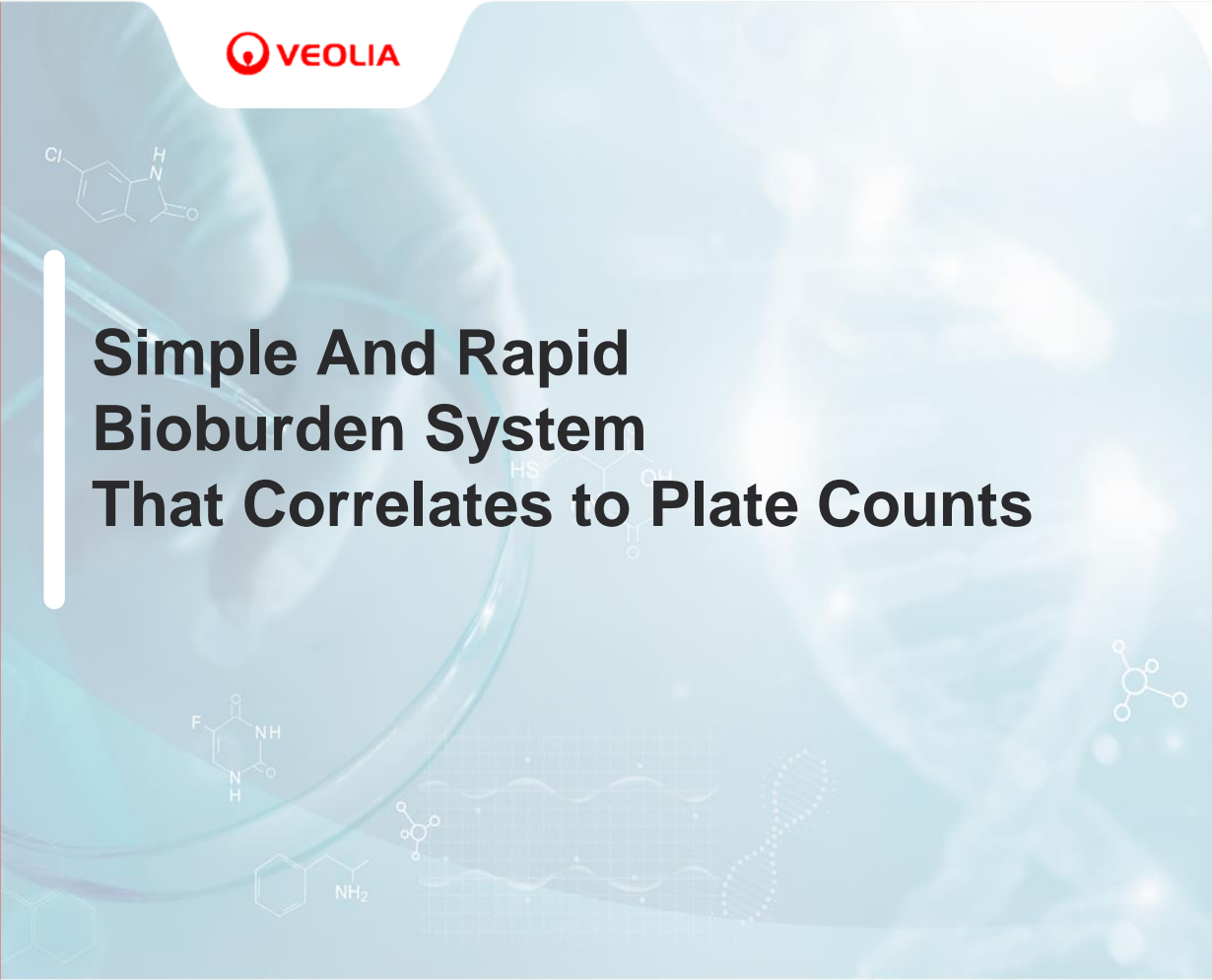


Isolate and Concentrate Target Analytes

Process on Bioburden Analyzer



SUMMARY

The background of the slide is a light blue, semi-transparent image of a hand holding a petri dish. Overlaid on this are various scientific and chemical symbols, including a DNA double helix, a chemical structure of a benzimidazole derivative, a chemical structure of a pyrimidine derivative, and a chemical structure of a benzene ring with an amino group. There are also some faint, abstract patterns and lines scattered across the background.

Simple And Rapid Bioburden System That Correlates to Plate Counts

You want more?

→ Find out more about
VEOLIA at SCC

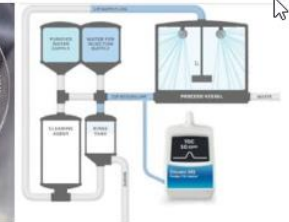
THANK YOU!

Questions?

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Zu den Produkten und Anwendungen im Bereich Reinigungsvalidierung, WFI- und HD-Wasserüberwachung usw. gehören:

- Gesamt organischer Kohlenstoff (TOC) Sensoren und Analysatoren (online-, portable- und labor-Systeme) – auch mit integrierten Leitfähigkeits-Sensoren
 - [SIEVERS CheckPoint TOC Sensor](#)
 - [SIEVERS online-, portable und labor M9 TOC Analysatoren](#)
 - [SIEVERS M500 Analysatoren](#)
- SIEVERS Plattform zum Testen bakterieller Endotoxine (BET)
 - [SIEVERS Eclipse BET Plattform](#)
- SIEVERS Keimbelastung (Bioburden) Schnelltestsystem
 - [SIEVERS Soleil System](#)
- [Zertifizierte Referenzmaterialien und Vials](#)
 - TOC-Standards ab 50 ppb und Leitfähigkeits-Standards ab 5 µS/cm
 - Vials
 - low-carbon TOC-Vials
 - vorgesäuerte TOC-Vials
 - vorgefüllte TOC-Vials für Reinigungsvalidierung
 - Kombinierte Leitfähigkeits- und TOC-Vials (DUCT)
- [SIEVERS Cloud-Lösung DataShare Elite Software](#)
- Validierungs-, Wartungs- und Applikations-Services



Alles selbstverständlich Pharmakopöe (z.B. EP/USP) konform, im Einklang 21 CFR Part 11 und Data Integrity (ALCOA+)