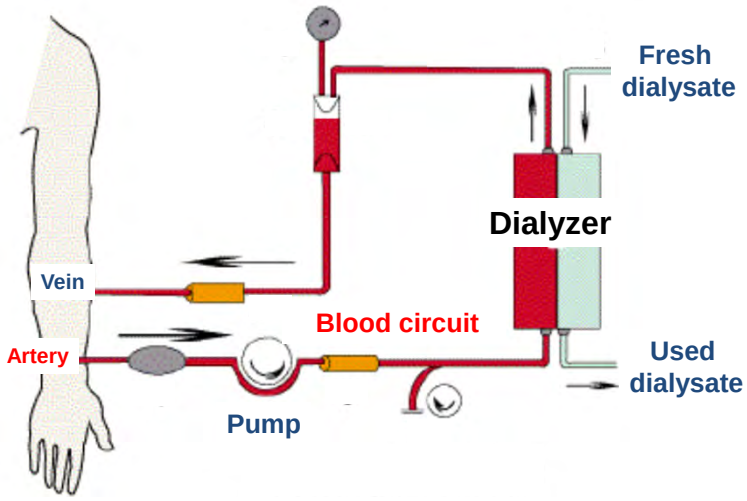
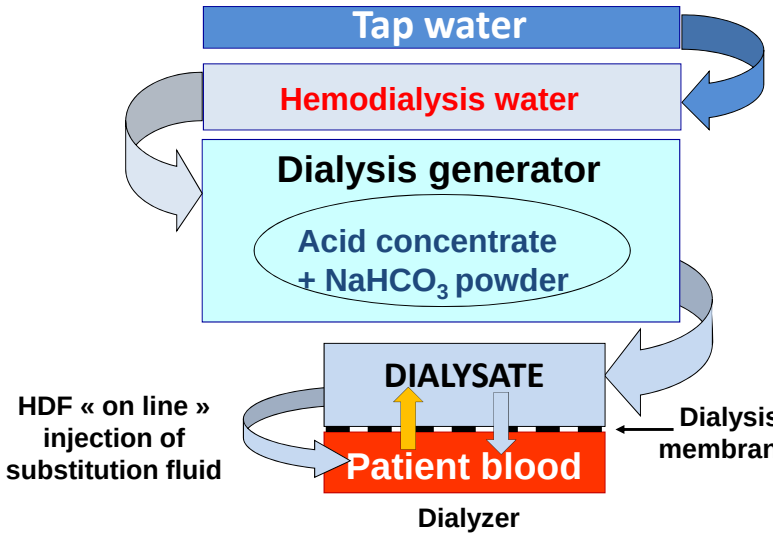
Treatment of chronic renal failure by hemodialysis techniques

Objective : Elimination of uremic toxins





Challenge

To produce from tap water high volumes of fluids (40 to 180 L/week) injected on line in the patient's blood

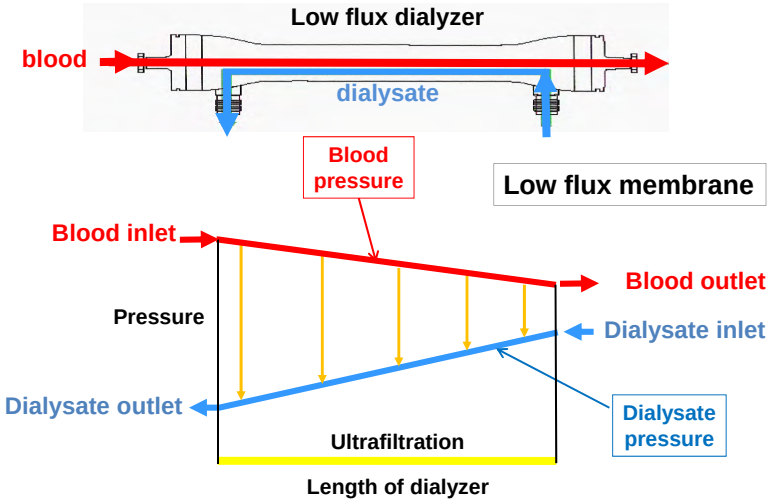
European Pharmacopoeia 9.0 – 2017

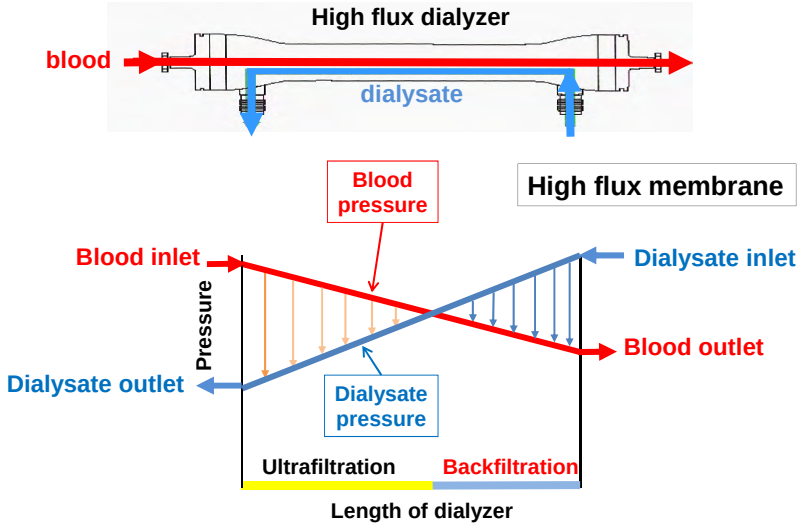
Monography of Water for haemodialysis

Definition

« Water for diluting concentrated haemodialysis solutions is obtained from potable water by distillation, by reverse osmosis, by ion exchange or by any other suitable method.

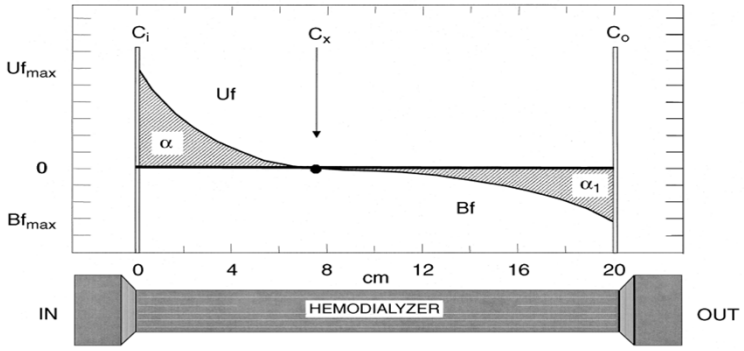
When water obtained by one of the methods described above is not available, potable water may be used for home dialysis »





Backfiltration up to 50 ml/min (3L/h) or 40L/week

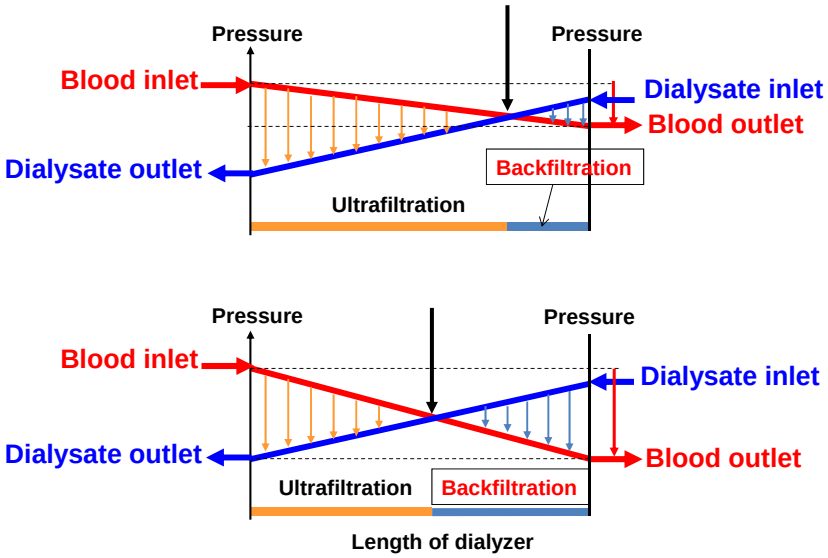
Yamashita AC et col – Am J Kidney Dis 2001; 38 (1):217-219



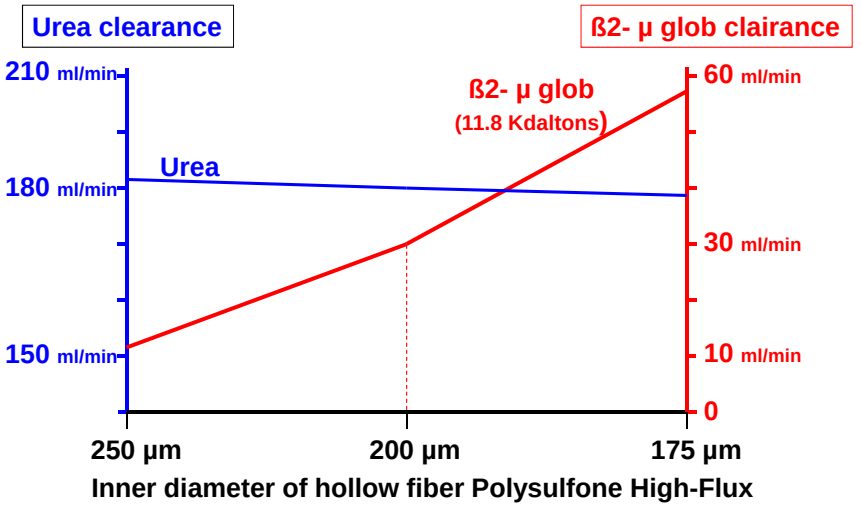
Ronco C et al: Hollow fibers in high-flux dialyzers
Kidney International, Vol. 58 (2000), pp. 809-817

The pressure profile along the hemodialyzer is not linear because of an increase of blood viscosity and oncotic power of plasma as water is removed by filtration in the first part of the hemodialyzer

Effects of a reduced inner diameter of hollow fibers in hemodialyzers



Effects of a reduced inner diameter of hollow fibers In hemodialyzers



Dellanna F et al NDT 1996; 11S2:83-6

Chemical quality of water

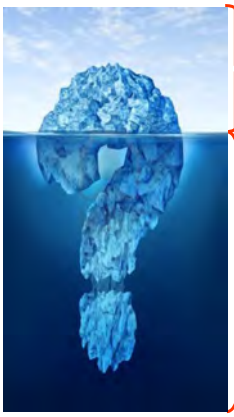
- ✓ **WFI : Sterile water for injection – Eur Pharm 2017 : 12 parameters**
- ✓ **Water for hemodialysis « on line » :**
  - **Eur Pharm 2017 : 16 parameters**
  - **ISO 23500 2015 : 22 parameters**

Microbiological quality of water

Maximum allowable levels for total viable microbial count and endotoxins in dialysis water

	Sterile WFI Eur Ph 2017	HD water Eur Ph 2017	HD water ISO 23500 : 2015
Bacteria	sterility	10^2 CFU / mL	100 CFU / mL
Endotoxins	0,25 UI / mL	0,25 UI/ mL	0,25 UI / mL

Microbiological HD water needs new methods for quality monitoring

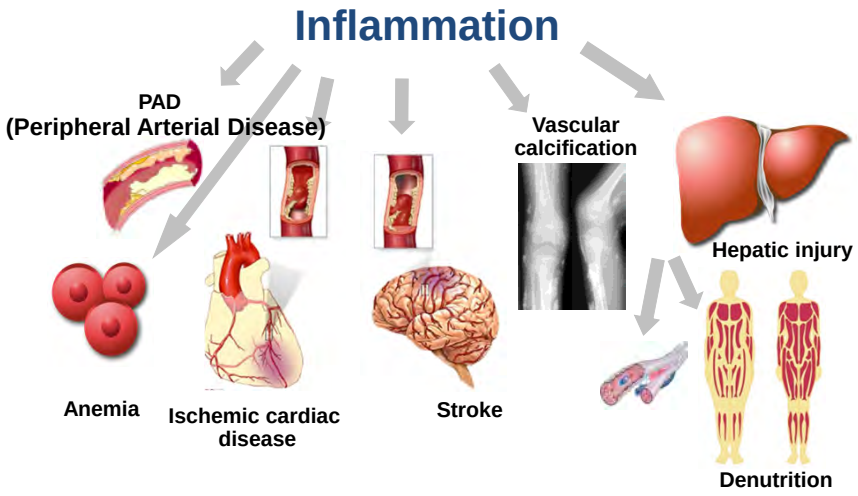


Detectable microbiological contaminants
- cultivable micro-organisms
- endotoxines

Non detectable contaminants
- non cultivable viable micro-organisms
- biofilm
- endotoxins fragments
- peptidoglycans
- DNA
- etc … can pass the dialysate membrane and induce inflammation

Recently the WHO published : « HPC represent 0.01 % of the total flora »

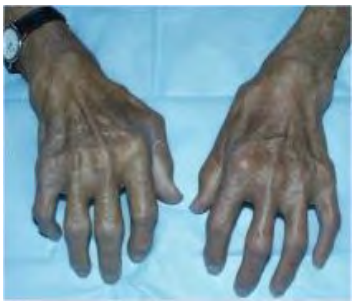
Impact of chronic inflammation in hemodialysis patients

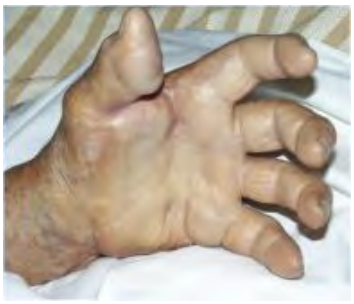


Carpal tunnel syndrome
An hemodialysis inflammatory iatrogenic pathology

Endotoxins induce the secretion of inflammation mediators : Interleukines, TNF α …

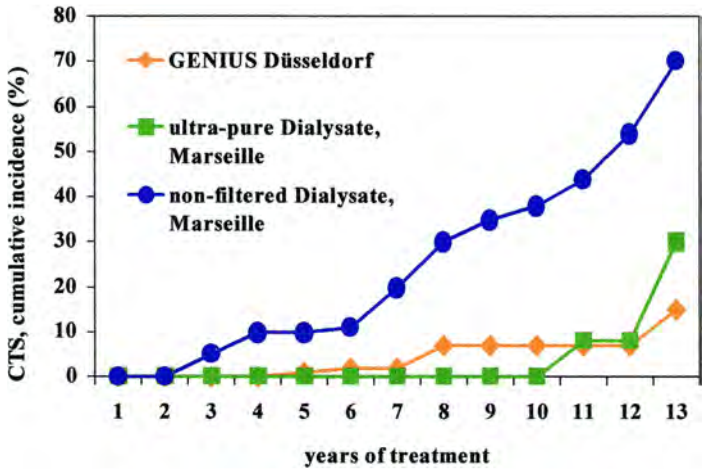
Stimulation and accumulation of β 2 microglobulin are responsible of amyloidosis





Photos Pr Bernard CANAUD

Ultrapure water in hemodialysis delays carpal tunnel syndrome



Lonnemann, G. et al. J Am Soc Nephrol 2002 ;13:72-S77

Dialysis fluid endotoxin level and mortality in maintenance hemodialysis

T.Hasegawa, S.Nakai, I.Masakane et al. Am J Kidney Dis. 2015;65(6):899-904

Cohort study on 130 781 patients – 98.9 % of dialysis centers in Japan

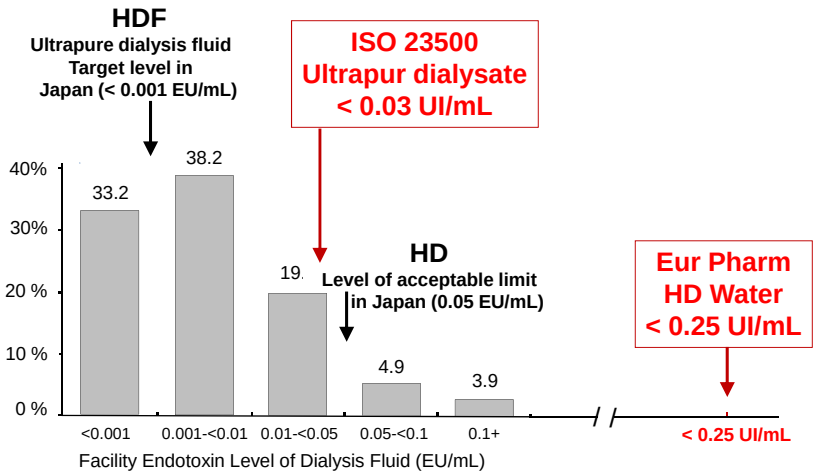
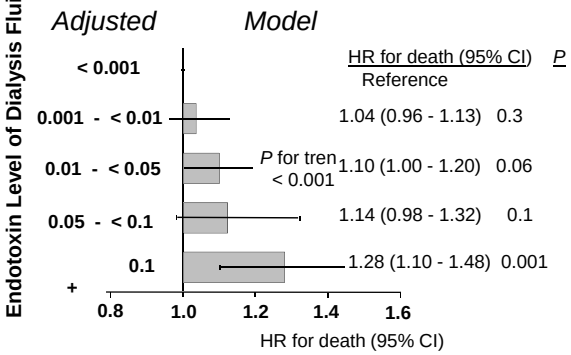


Figure 2. Distribution of facility dialysis fluid endotoxin levels. Data relate to in-center hemodialysis patients in Japan.

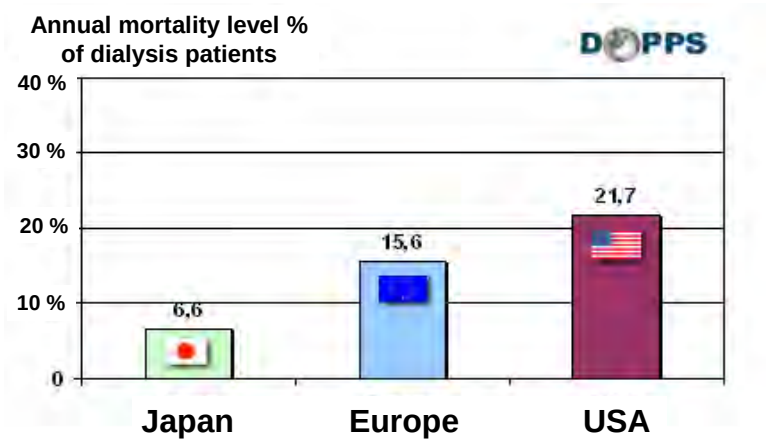
Relation between mortality risk and endotoxins levels



Mortality risk and endotoxins levels	
< 0.001	0%
0.001 to < 0.01	4%
0.01 to < 0.05	10%
0.05 to < 0.1	14%
0.1 and >	28%

Figure 3. Hazard ratio (HR) of all-cause mortality for in-center hemodialysis patients stratified by facility dialysis fluid endotoxin level ( ) adjusted for age, sex, dialysis vintage diabetes mellitus, Kt/V, normalized protein catabolic rate, dialysis sessions duration, serum albumine level, hemoglobin level

T.Hasegawa, S.Nakai, I.Masakane et al. Am J Kidney Dis. 2015;65(6):899-904



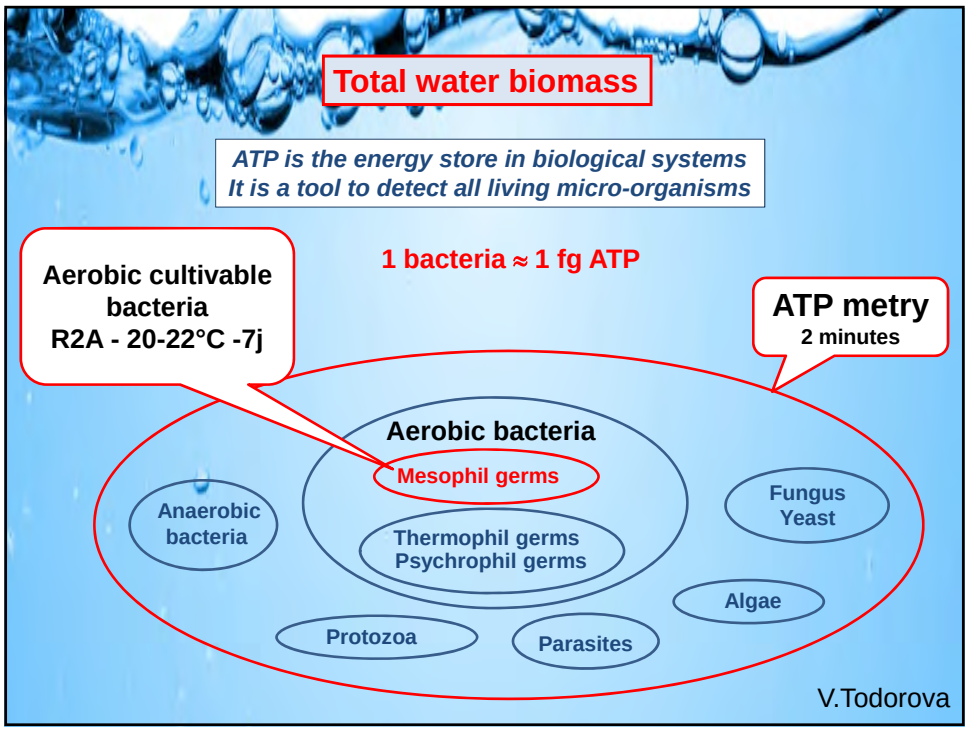
Endotoxins levels in water > 0,1 UI/ml = increase of 20% of mortality risk

Masakane Ikuto ASN 2008

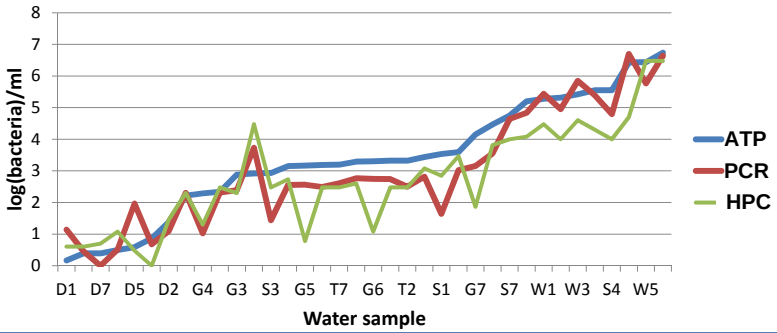
Overview of methods used for general bacteria contamination in water

Method	▪ Measures ▪ Principle	Labor	Time to result	On line	Cost
HPC Heterotrophic Plate Counts	Cultivable bacteria Growth	Low	Days to weeks	No	Low
Endotoxins	LAL Test Bacteria gram –	Medium	Minutes to yours	No	High
Microscopy DAPI, FISH	Cell concentration	High	Minutes to hours	No	Medium
FCM Flow cytometry	Cell concentration	Low	Minutes	Yes	High
ATPmetry	ATP concentration Enzymatic	Low	Minutes	Yes	Low
qPCR	16S rRNA Gene copies Gene amplification	High	Hours to days	No	High
Nucleic acid quantification	Total DNA/RNA	High	Hours to days	No	High

Modified from S.Van Nevel et al. / Water Research 113 (2017) 191 - 206



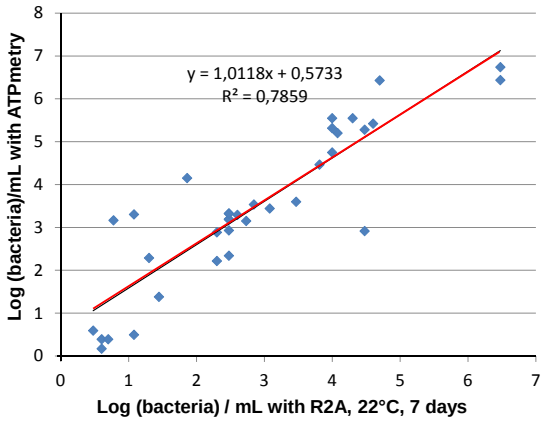
Is there a correlation between HPC (Heterotrophic Plate Counts) and new alternative methodology to estimate total flora in water ?



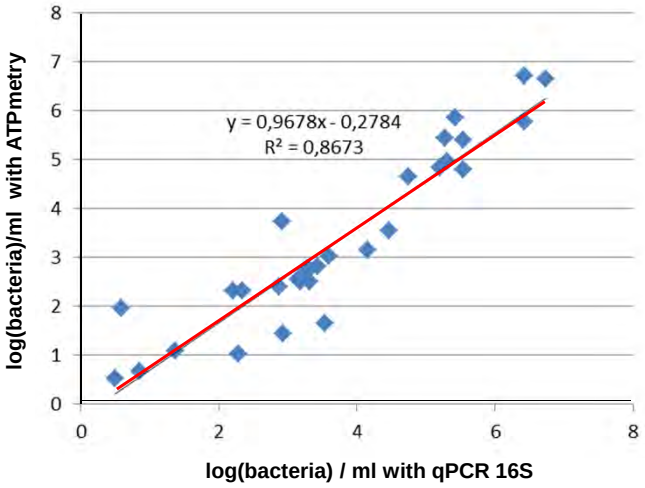
D = drinking water G = ground water T = treated water S = surface water W = waste water

For different qualities of water the global estimation of the microbiological biomass is similar wathever the analytical technics

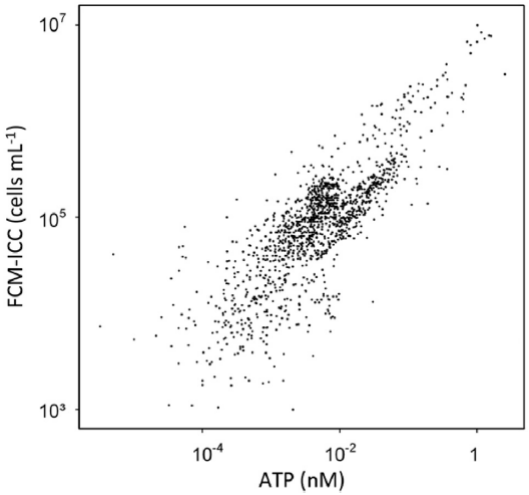
Correlation between ATPmetry and HPC (R2A, 22°C, 7 days)



Correlation between ATPmetry and qPCR 16S

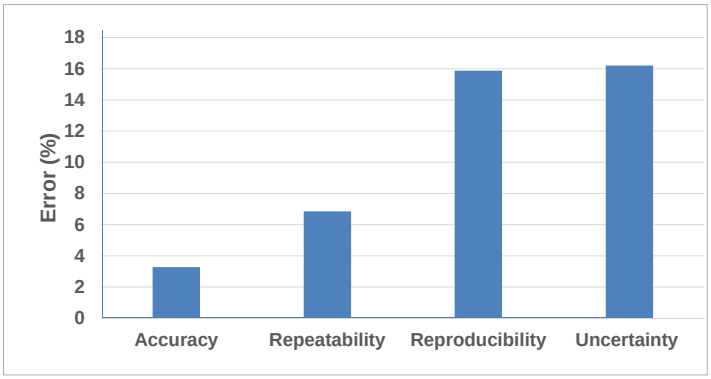


Correlation between ATPmetry and FCM-ICC

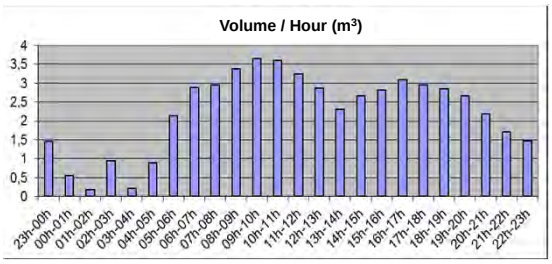


S.Van Nevel et al. / Water Research 113 (2017) 191 - 206

Performance of GLBiocontrol ATPmetry



Limit of quantification : 1 pg ATP/Liter
Limit of detection : 0.1 pg ATP/Liter ≈ 100 bacteria / Liter

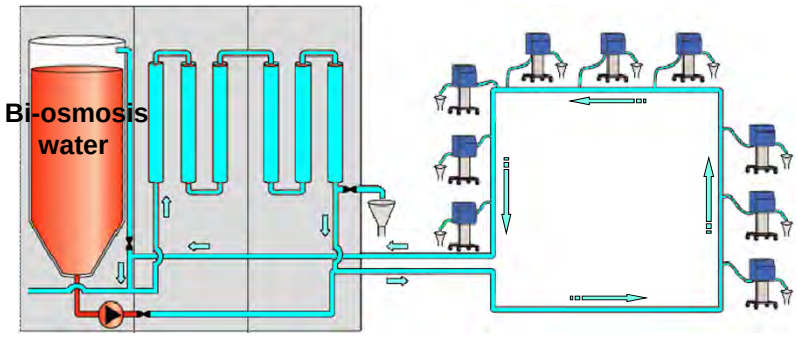


HDF on line
76 generators

54 m³ water / day

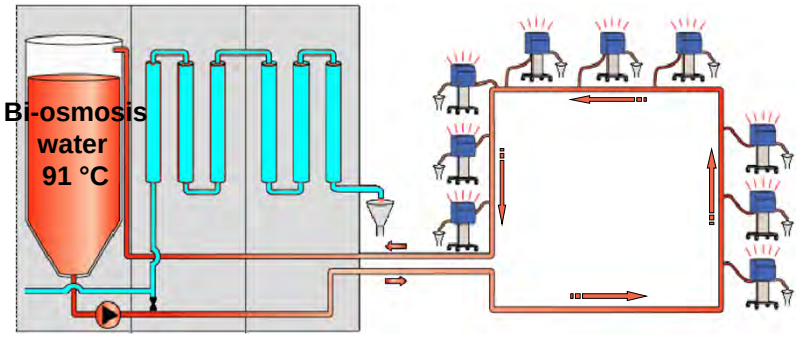
≈ 300 L / session

Thermic disinfection with hot water
of hemodialysis water distribution loop



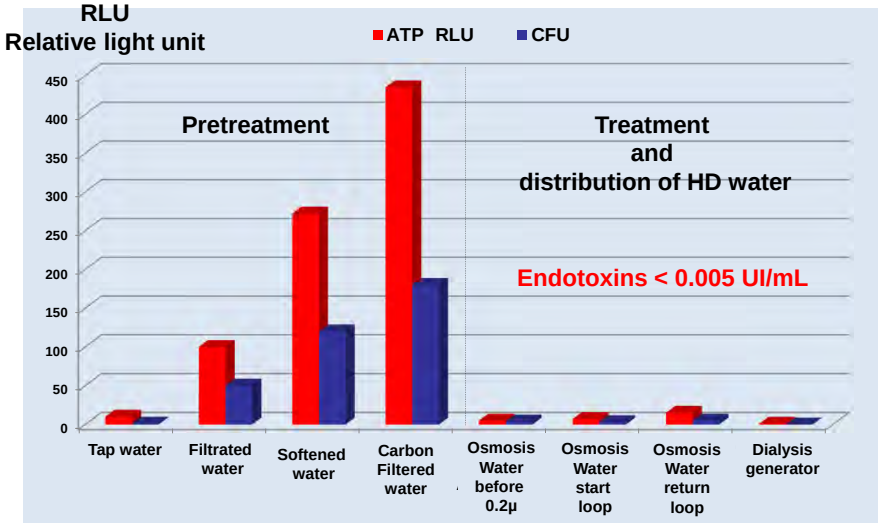
HD Center – University hospital of Marseille – France

Thermic disinfection with hot water
of hemodialysis water distribution loop



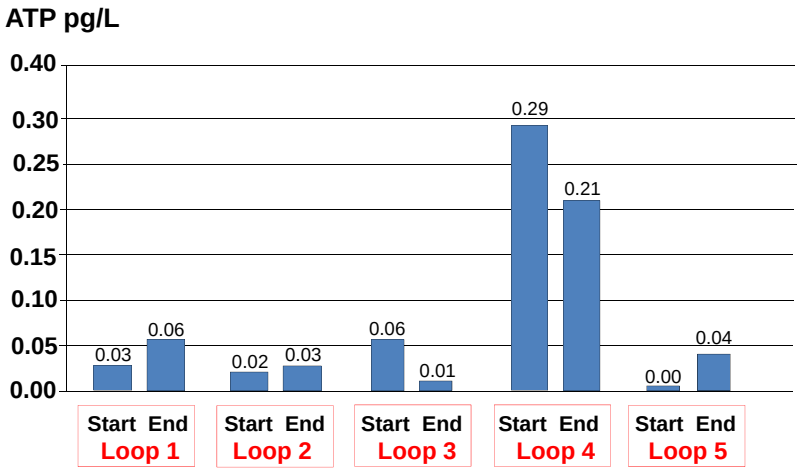
HD Center – University hospital of Marseille – France

Comparison ATP metry / HPC (Heterotrophic Plate Count)
in a water treatment unit to produce and distribute HD water



HD Center – University hospital of Marseille – France

Concentrations of ATP (pg/Liter)
in the 5 distribution loops of hemodialysis water

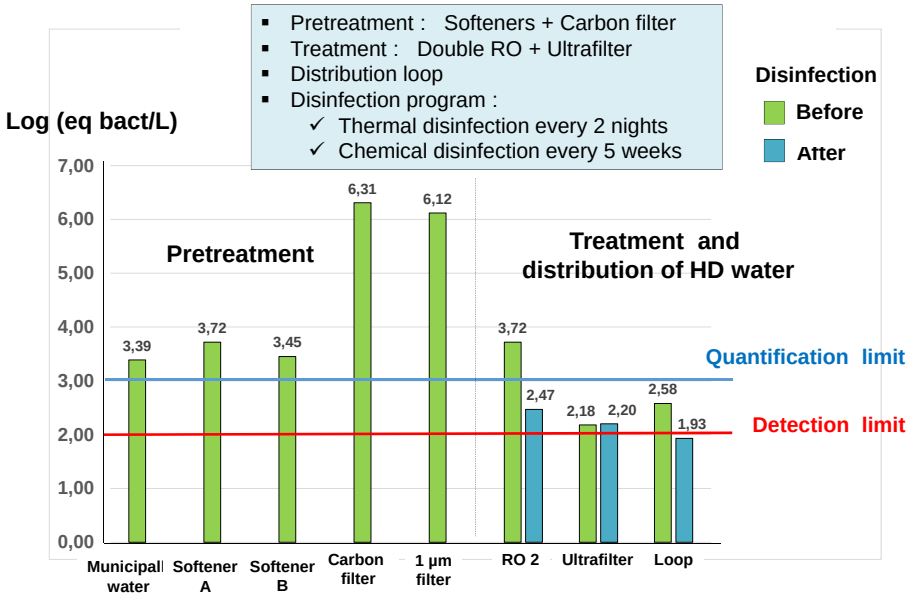


0.1 pg ATP / L ≈ 100 bact /L

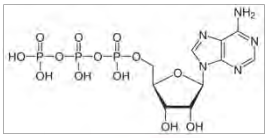
HD Center – University hospital of Marseille – France

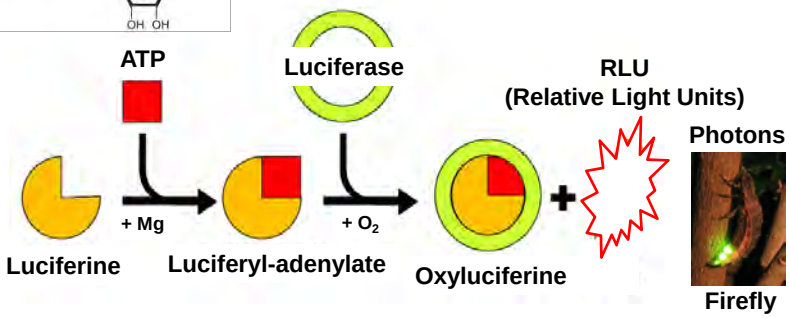
ATP levels along an hemodialysis water treatment

- Pretreatment : Softeners + Carbon filter
- Treatment : Double RO + Ultrafilter
- Distribution loop
- Disinfection program :
  - ✓ Thermal disinfection every 2 nights
  - ✓ Chemical disinfection every 5 weeks



Bioluminescence reaction





Quantitative ATPmetry

Required equipment for ATPmetry

- ✓ **Sterile single use devices**
  - Seringe
  - Tubes
  - Microfilter (0.45 µm)

- ✓ **Reagents**
  - Enzymatic
  - Standard for calibration

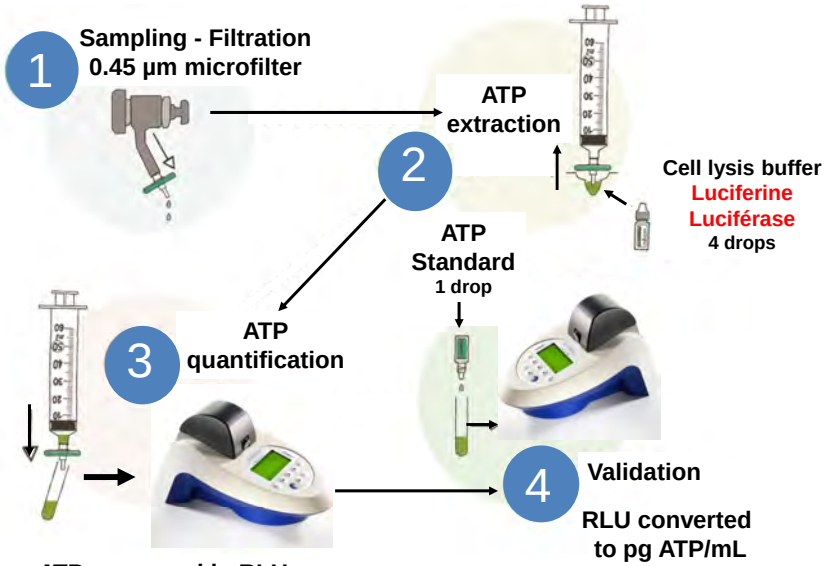
- ✓ **Luminometer**

Laboratory

or

Field

ATPmetry protocol



ATPmetry advantages

ATP on filter is an efficient methodology to estimate total flora

- ✓ **Rapid : < 2 minutes**
- ✓ **Easy to use : protocol in 4 steps**
  - **Filtration**
  - **Extraction of ATP**
  - **Quantification**
  - **Validation**
- ✓ **Calibrated in each sample with internal standard**
- ✓ **Quantification : linearity range from 1 pg/L to 10^6 pg/L**
- ✓ **Field compatible**
- ✓ **Automatable**

CONCLUSION

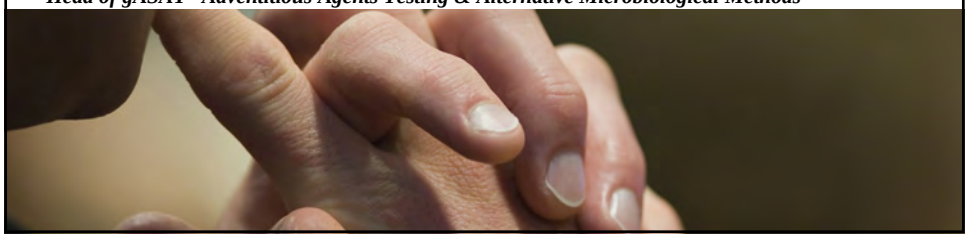
- ✓ **ATPmetry is a standard tool for measuring the total biomass in water**
- ✓ **It would replace with benefit the cultivable methods to :**
  - **to determine rapidly and with trust the microbiological quality of the water delivered to dialysis patient**
  - **to validate and monitor the disinfection programs.**
- ✓ **The monitoring of the microbiological contamination of HD water with ATPmetry is :**
  - **to demonstrate that the disinfection program is effective**
  - **not to indicate when disinfection should be performed**

Thank you for your attention

Online Bioburden Monitoring of Water Systems – Feasibility Studies

International Microbiology Symposium
October 10-11, 2017, EDQM, Strasbourg, France

Dr. Sven M. Deutschmann, Roche Diagnostics GmbH, Director QC Pharma Biotech Penzberg
Head of gASAT "Adventitious Agents Testing & Alternative Microbiological Methods"



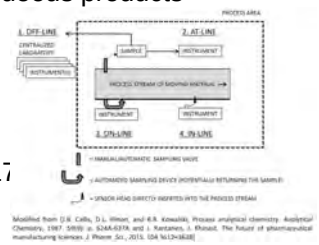
Introduction

Feasibility Studies

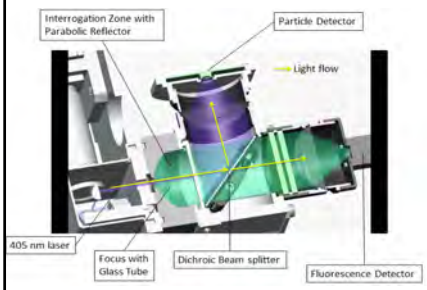
Online Bioburden Monitoring of Water Systems
Technology (1)

What's about the new technology?

- Non-growth–based detection of waterborne microbes
- non-destructive technology
- readout: optical sensor
- minimized human interventions
- real-time microbial and particle analyzer for aqueous products
- continuous monitoring
  - corrective measures: short reaction time
- at-line measurement
  → note: per Ph. Eur. draft 5.25
  "Process analytical technology" (March 2017



Online Bioburden Monitoring of Water Systems
Technology (2)

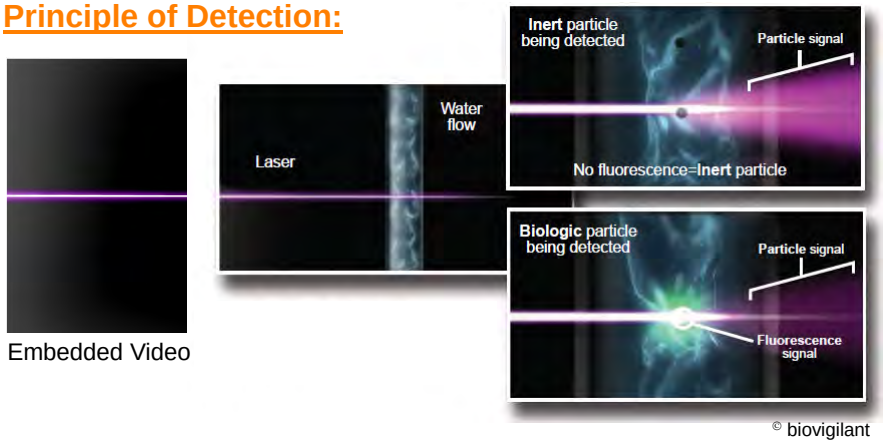


Detection method:

- Flow cytometric approach with two synchronized methods:
  - **Mie Scattering** for measuring the particle size: larger particles result in more intensive scattering.
  - **Intrinsic fluorescence (autofluorescence)** for differentiating viable from inert microbes: metabolites of viable microbes e. g. NADH / riboflavin result in fluorescent signals.
- software combines the data and differentiates between inert particles and biological cells.

Online Bioburden Monitoring of Water Systems
Technology (3)

Principle of Detection:



Embedded Video

© biovigilant

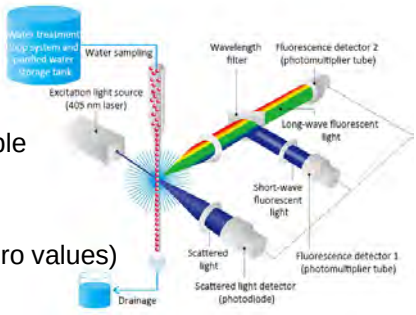
Online Bioburden Monitoring of Water Systems
Technology (4)

Technology-Generations:

1st generation: using the light scattering and one fluorescent channel

2nd generation: using the light scattering and two fluorescent channels

- Two photomultiplier tube (PMT)
  - short wave fluorescence
  - long wave fluorescence
- Provides better differentiation of viable and non-viable fluorescing particles
  → reduced background noise
  → increased sensitivity (in theory: zero values)



Introduction

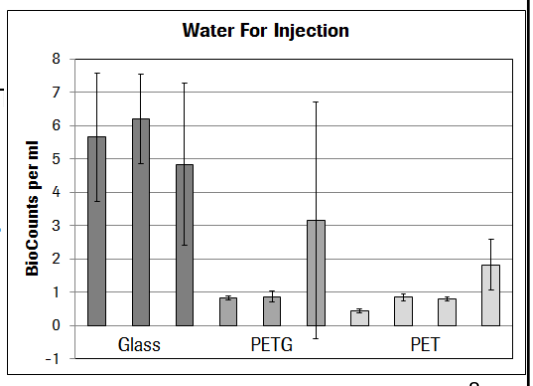
Feasibility Studies

Online Bioburden Monitoring of Water Systems
Feasibility Study – 1st Generation (1)

Sampling Devices – Particle Shedding (1):

- 250 mL sampling bottles
- Different material:
  - glass
  - Polyethylenterephthalat (PE
  - PET with glycerine coating
- Water source =WFI

➢ **PET- or PETG-bottles better suited for grab sampling**



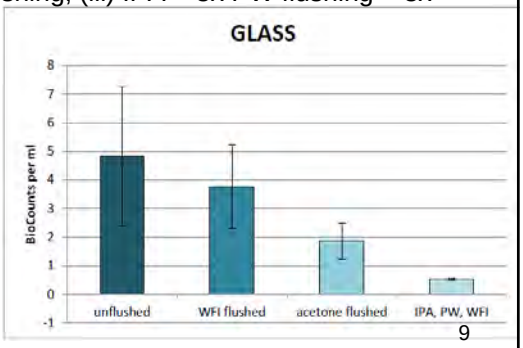
Online Bioburden Monitoring of Water Systems
Feasibility Study – 1st Generation (2)

Sampling Devices – Particle Shedding (2):

- 250 mL glass sampling bottles as worst case
- Pre-treatment of the glass bottles: (i) 3x WFI flushing, (ii) acetone flushing + 3x WFI flushing, (iii) IPA + 3x PW flushing + 3x WFI flushing

➢ **Pre-treatment of bottles can reduce particle shedding**

➢ **Note: used bottles will shed more particles**



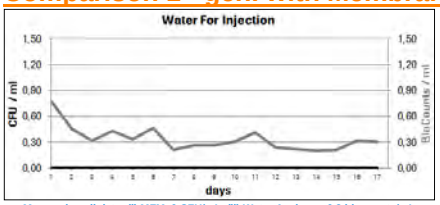
Online Bioburden Monitoring of Water Systems
Feasibility Study – 1st Generation (3)

Comparison 1st gen. with Membrane Filtration – Experimental Setup:

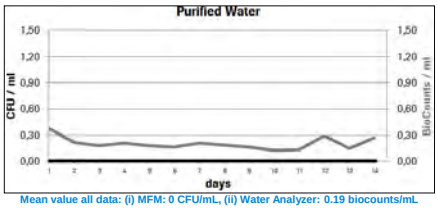
- 250 mL PETG sampling bottles
- 100 mL testing volume
- Water sources:
  - WFI
  - Purified Water
  - Tap Water
- Membrane filtration method (MFM) with >5 d incubation at 30 – 35 °C
- Results per day consist of 6 replicates, each

Online Bioburden Monitoring of Water Systems
Feasibility Study – 1st Generation (4)

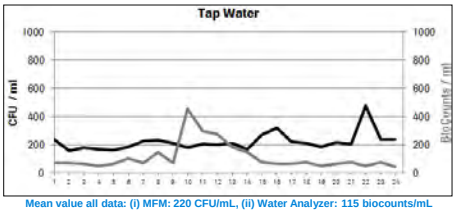
Comparison 1st gen. with Membrane Filtration – Results (1):



Mean value all data: (i) MFM: 0 CFU/mL, (ii) Water Analyzer: 0.3 biocounts/mL



Mean value all data: (i) MFM: 0 CFU/mL, (ii) Water Analyzer: 0.19 biocounts/mL



Mean value all data: (i) MFM: 220 CFU/mL, (ii) Water Analyzer: 115 biocounts/mL

Note:
black line = membrane filtration / CFU/mL
Grey line = water analyzer / biocounts/mL

Online Bioburden Monitoring of Water Systems
Feasibility Study – 1st Generation (5)

Comparison 1st gen. with Membrane Filtration – Results (2):

➢ **Biocounts ≠ Colony Forming Units !**

1. WFI
   - higher biocount values compared to Purified Water, due to
     - high background noise
     - Resulting in false positive biocount values
2. Purified Water
   - Biocount values are quiet reasonable
3. Tap Water
   - Biocount values seem to be low (compared to other experimental resuts)
   - High variation between different experiments, dependent on
     - amount of microorganisms
     - growth rate
     - weather conditions

Online Bioburden Monitoring of Water Systems
Feasibility Study – 2nd Generation (1)

Comparison 2nd gen. with Membrane Filtration – Experimental Setup:

- Sampling and dilution in unrinsed PETG-bottles
- 20 mL aliquots of tap water
- Frozen at -20 °C
- Day 1, 2 and 3 thawed and diluted with WFI (total volume: 2000 mL)
- mixture was aliquoted into 14 samples
  - 7 samples for membrane filtration (6d incubation at 30 – 35 °C)
  - 7 samples for the 2nd gen. water analyzer

RESULTS:

	June 20	June 21	June 22
2nd gen. water analyzer / [mean biocounts/mL]	50.1	90.9	69.8
membrane filtration / [mean CFU/mL]	2.1	2.9	3.7
Ratio biocount:CFU	23.7	31.8	19.0

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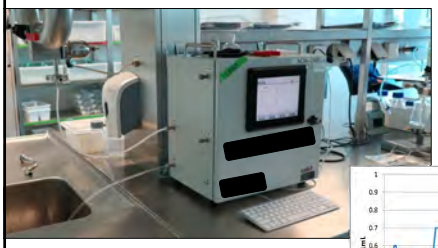
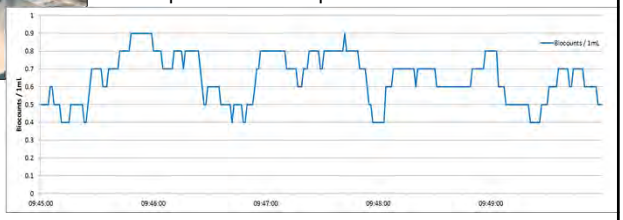
Feasibility Study – 2nd Generation (2)

Monitoring of a Purified Water System:

The water analyzer was connected to the Purified Water 2 loop in the lab with the provided 1/4'' PTFE tubing.



Left: Experimental Setup



Right: Results

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Feasibility Study – 2nd Generation (3)

Monitoring of a WFI Water System – Experimental Setup:

1/4'' PTFE tubing was used to connect the machine to the valve of the WFI1 loop. In order to cool the WFI from above 80°C at the valve to below 60°C for the IMD-W, two cooling spirals were built in after which the tubing passes through a water bath for further cooling down. This way the water could be cooled below 30°C.

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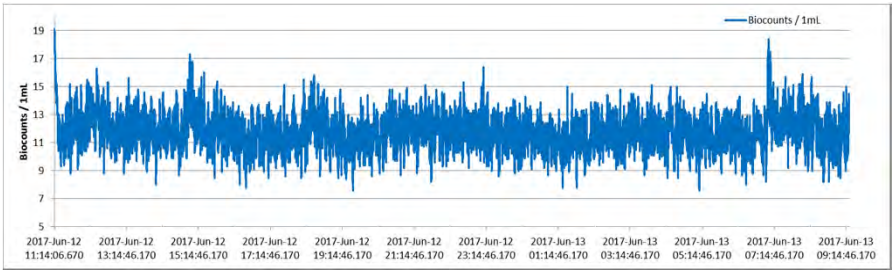
Feasibility Study – 2nd Generation (4)

Monitoring of a WFI Water System - Results



Online Bioburden Monitoring of Water Systems
Feasibility Study – Summary & Conclusion

PROS Water Analyzer

- Fast & non-growth method
- Easy to handle
- No aseptic working necessary
- Electronic data sets are created
- Sample and online mode possible
- Sensitivity is sufficient

CONS Water Analyzer

- No common approved method
- No identification possible
- Lack of robustness (suddenly occurring technical issues / troubles)

Conclusion:

- **Biocounts ≠ Colony Forming Units !**
- **The user must generate a new baseline for the water system**

➢ **Can be used for the detection of changes of a water quality in real-time**

Online Bioburden Monitoring of Water Systems
Acknowledgements

Séverine Olivia Baur, Master Student (1st gen. study)
Caroline Schmitt-Koopmann, Master Student (2nd gen. study)
Daniel Kockelkorn, Roche (Kaiseraugst)
Christian Siegmund, Roche (Kaiseraugst)
Ulrich Georg Zuber, Roche (Kaiseraugst)

Online Bioburden Monitoring of Water Systems

Q & A

Thank you for your attention !

Doing now what patients need next

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Rapid Microbiological Methods (RMM) and Process Water Quality

An Australian Perspective

Karen Longstaff
Director, Microbiology Section
Laboratories Branch
Medical Devices and Product Quality Division, TGA

TGA Health Safety Regulation

EDQM International Microbiology Symposium
11 October 2017

Australian Government
Department of Health
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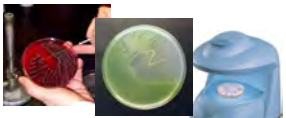
Presentation Scope

- Provide an overview of current RMM approved in Australia for testing of process waters
- Identify benefits of RMM versus traditional microbiological methods
- Address major challenges for regulatory approval
- Tips for applicants



RMM: TGA's Experience

- TGA acknowledges and is supportive of new technologies
  - Legislation has no mechanism to provide approval for equipment
  - Approvals are based on individual finished product registration
- Companies do consider using RMM
- Some discuss their intentions with TGA:
  - We encourage adoption of RMM
  - We discuss regulatory expectations on case-by-case basis
- Validation RMM proceeds
- Company continues with compendial method
- Why?

Commonwealth Department of Health

- **Code of Good Manufacturing Practice for Therapeutic Goods (1970):**

- **11.2** ***Water for parenteral products:***
  - Prepared by distillation
  - Meet BP WFI quality criteria
  - Minimal time between distillation and product sterilization
  - No storage water or product prior to sterilization
- **14.** ***Quality control:***
  - **14.2** Test for pyrogens shall be carried out regularly on randomly selected parenteral products or on the water used in such products

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Commonwealth Department of Health

- **Code of Good Manufacturing Practice for Therapeutic Goods (1983):**

- ***Part 1 Finished Dosage Forms:***
  - *6.8 Contamination control:*
    - 6.8.2.4 Frequent microbiological monitoring of process water, including at point of use, ensuring sample size and test method can detect presence of low levels of indicator organisms, e.g. pseudomonads.
- ***Part 2 Sterile Products:***
  - 16.2 *Water for parenteral products:*
    - As per 1970 edition
    - Monitor weekly for microbial contamination
    - Held at ≥ 80°C or drained at end of day

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Department of Health
Therapeutic Goods Administration

Commonwealth Department of Community Services and Health

- **Australian Code of Good Manufacturing Practice for Therapeutic Goods (1990):**

- ***Part 1 Medicinal Products (c635-639):***
  - Recognizes process water:
    - Critical starting material
    - Source of contamination
  - Requires:
    - Suitable design, validation and control of water system
    - Chemical and microbiological control:
      - Tested 'sufficiently frequently to demonstrate system is in control'
    - Purified water point of use action level of 10^2 CFU/mL
- ***Part 2 Sterile Products:***
  - c1502-1505 essentially as per 1983

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Australian Code of Good Manufacturing Practice for Therapeutic Goods (1990)

- **Appendix F *Guidelines for estimation of microbial count in process water:***

- Note: Australian Code GMP superseded by PIC/S Code in 2002

- Two methods:
  - Spread/pour plate
  - Membrane filtration
- 'Suitable agar':
  - Not specified
- Incubation period:
  - 5 days
- Incubation temperature:
  - Not specified
  - 'Temperatures significantly above 30°C may give poor recoveries'

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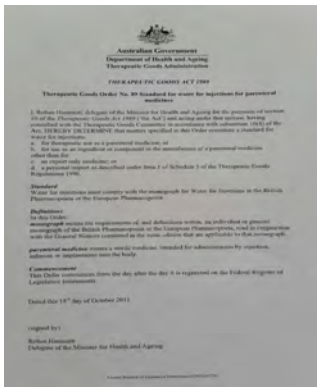
Therapeutic Goods Order No.89

- ***Standard for water for injections for parenteral medicines (2011):***
  - WFI must comply with the Ph. Eur. or BP monographs
  - Including General Notices applicable to monographs:
    - *Permits alternative methods of analysis, e.g. RMM*
  - Monograph test method:
    - Membrane filtration
    - R2A agar
    - Incubate 30°-35°C for ≥ 5 days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THERAPEUTIC GOODS ACT 1989

Therapeutic Goods Order No. 89 Standard for water for injections for parenteral medicines

RMM – Process Waters

- Technical benefits:
  - Faster time to result
  - Improve quality of microbiological testing
    - Accuracy, sensitivity and specificity
    - Less repeat testing
  - Improve process control and quality control:
    - Real-time/near real-time counting of process water monitors quality during product manufacture not after the event
    - Respond earlier to excursions and adverse trends
    - Implement investigative and corrective actions earlier
  - Automate aspects of testing:
    - Direct capture of test data
  - Professional development of analysts

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RMM – Process Waters

- Financial/business benefits:
  - Complements process and product quality risk management:
    - Potentially reduce risk of product contamination
    - Continual improvement
  - Reduce production delays:
    - Reduce need to reject product or recall product
  - Faster product release
  - Cost savings:
    - Labour/analyst efficiencies, time, production, warehousing

RMM – Process Waters

- Adoption of RMM:
  - Endotoxin detection
  - Organism identification:
    - MALDI-TOF (matrix assisted laser desorption ionisation time- of-flight)
    - MicroSEQ® Rapid Microbial Identification System
    - Whole genome sequencing (reference laboratory):
      - Phylogenetic analysis of outbreak clusters

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RMM – Process Waters

- **Why the reluctance to farewell the traditional agar plate and CFU per volume tested?**

Reluctance to change from compendial method

- Access to relevant microbiological expertise:
  - Limited or no expertise on site
  - Samples ➡ contract testing laboratory
  - Contract testing laboratory uses compendial method
- High initial costs for RMM:
  - Complex technological platforms and sophisticated equipment:
    - More complex than filtration, plate count, incubator and colony counter
  - Validation:
    - Whole system, software, microbiological performance
    - Validation effort and possible challenge by Regulator might be a barrier
  - Is the cost of an RMM for process water justifiable if there is a 5-7 day wait for a microbial limits test result or a 14 day wait for a test for sterility result?
  - Low sales volume for inexpensive product

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Reluctance to change from compendial method

- Not willing to be the first to ‘dip toes into the water’

- Might exceed limits so historical trends are affected:
  - Doesn’t necessarily mean new quality/safety risks exist
- Current method is ‘cheap and adequate for the job’:
  - Is it really?
  - Non-sterile oral hygiene product:
    - Colonisation/infection of ICU patients with *Burkholderia cepacia* complex (BCC)
    - Implicated batch contaminated with 10^5 to 10^6 CFU/g of BCC
    - Consumer level recall
  - Sterile ultrasound gel:
    - Infection of ICU patients with BCC
    - Implicated batch contaminated with average 2.6 x 10^4 CFU/g of BCC
    - Hospital level recall
  - Process water might have been the contamination source

Summary

- Pharmaceutical industry has a conservative culture:
  - Risk-averse
  - Possibly stifled adoption of RMM
- Recognition that timely microbiological data is vital for:
  - Process monitoring and control
  - Product release
- Now more awareness of need to consider risk benefit offered by RMMs in terms of:
  - Business risk and management of product quality
  - Cost/savings over the long term
- Important to identify user requirements and determine how these can be met:
  - Work closely with RMM suppliers, technical advisors, and regulators
  - Equipment selection, validation, documentation, training, maintenance, ongoing support etc.
- Consider taking the plunge

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