Interest of ATPmetry for the microbiological control of haemodialysis water

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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 »
Ultrafiltration
Pressure
Low flux membrane
Low flux dialyzer
Blood pressure
Length of dialyzer
Dialysate inlet
Dialysate outlet
Blood inlet
Blood outlet
Dialysate
Blood
dialysate

Blood inlet
Blood outlet
Pressure
Dialysate inlet
Dialysate outlet
Ultrafiltration
Length of dialyzer
Blood pressure

Blood inlet
Blood outlet
Pressure
Dialysate inlet
Dialysate outlet
Ultrafiltration
Backfiltration
Length of dialyzer
Blood pressure

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

**Urea clearance**

- 210 ml/min
- 180 ml/min
- 150 ml/min

**β2-µglobulin clearance**

- 60 ml/min
- 30 ml/min
- 10 ml/min

Inner diameter of hollow fiber: Polysulfone High-Flux

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

<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Bacteria sterility</td>
<td>10^2 CFU / mL</td>
<td>100 CFU / mL</td>
<td>100 CFU / mL</td>
</tr>
<tr>
<td>Endotoxins</td>
<td>0.25 UI / mL</td>
<td>0.25 UI / mL</td>
<td>0.25 UI / mL</td>
</tr>
</tbody>
</table>
Detectable microbiological contaminants
- cultivable micro-organisms
- endotoxins

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

Inflammation
- PAD (Peripheral Arterial Disease)
- Anemia
- Ischemic cardiac disease
- Vascular calcification
- Hepatic injury
- Denutrition
- Stroke
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

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

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.
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

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

Modified from S. Van Nevel et al. / Water Research 113 (2017) 191 - 206
ATP is the energy store in biological systems. It is a tool to detect all living micro-organisms.

1 bacteria = 1 fg ATP

Aerobic cultivable bacteria
R2A - 20-22°C -7j

ATP metry
2 minutes

Total water biomass

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

For different qualities of water the global estimation of the microbiological biomass is similar whatever the analytical technics.
Correlation between ATPmetry and HPC (R2A, 22°C, 7 days)

\[ y = 1.0118x + 0.5733 \]
\[ R^2 = 0.7859 \]

Correlation between ATPmetry and qPCR 16S

\[ y = 0.9678x - 0.2784 \]
\[ R^2 = 0.8673 \]
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 \approx 100\ bacteria / Liter
Nephrology - Hemodialysis center
University Hospital of Marseille - France

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

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

Endotoxins < 0.005 UI/mL
Concentrations of ATP (pg/Liter) in the 5 distribution loops of hemodialysis water

ATP pg/L

<table>
<thead>
<tr>
<th>Loop</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
<th>End</th>
<th>Start</th>
<th>End</th>
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<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.06</td>
<td></td>
<td>0.02</td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.06</td>
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<tr>
<td>2</td>
<td>0.01</td>
<td></td>
<td>0.03</td>
<td></td>
<td>0.06</td>
<td></td>
<td>0.06</td>
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<td>0.01</td>
<td></td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.20</td>
<td></td>
<td>0.25</td>
<td></td>
<td>0.29</td>
<td></td>
<td>0.21</td>
<td></td>
<td>0.20</td>
<td></td>
<td>0.20</td>
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<tr>
<td>4</td>
<td>0.40</td>
<td></td>
<td>0.30</td>
<td></td>
<td>0.10</td>
<td></td>
<td>0.05</td>
<td></td>
<td>0.00</td>
<td></td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td></td>
<td>0.02</td>
<td></td>
<td>0.06</td>
<td></td>
<td>0.01</td>
<td></td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

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

Log (eq bact/L)

Pretreatment

- Municipal water
- Softener A
- Softener B
- Carbon filter
- 1 µm filter

Treatment and distribution of HD water

- RO 2
- Ultrafilter
- Loop

Disinfection

Quantification limit

Detection limit
Bioluminescence reaction

ATP $+ \text{Mg} \rightarrow$ Luciferine $\rightarrow$ Luciferyl-adenylate $+ O_2 \rightarrow$ Oxyluciferine $\rightarrow$ Photons

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

1. Sampling - Filtration
   0.45 µm microfilter

2. ATP extraction
   Cell lysis buffer
   Luciferine
   Luciférase
   4 drops

3. ATP quantification
   ATP Standard
   1 drop

4. Validation
   RLU converted to pg ATP/mL
   (1pg ATP ≈ 1000 bacteria)

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 10⁶ pg/L
- Field compatible
- Automatable
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”

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**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)

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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:**

[Image of Principle of Detection]

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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 (PET)
  - 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

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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
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Feasibility Study – 1st Generation (4)

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

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

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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 results)
   - High variation between different experiments, dependent on
     - amount of microorganisms
     - growth rate
     - weather conditions
Comparison 2nd gen. with Membrane Filtration – Experimental Setup:
- 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:

<table>
<thead>
<tr>
<th></th>
<th>June 20</th>
<th>June 21</th>
<th>June 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd gen. water analyzer / [mean biocounts/mL]</td>
<td>50.1</td>
<td>90.9</td>
<td>69.8</td>
</tr>
<tr>
<td>membrane filtration / [mean CFU/mL]</td>
<td>2.1</td>
<td>2.9</td>
<td>3.7</td>
</tr>
<tr>
<td>Ratio biocount:CFU</td>
<td>23.7</td>
<td>31.8</td>
<td>19.0 13</td>
</tr>
</tbody>
</table>

Online Bioburden Monitoring of Water Systems
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.
Monitoring of a WFI Water System – Experimental Setup:

1/4” PTFE tubing was used to connect the machine to the valve of the WFI 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.
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

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Christian Siegmund, Roche (Kaiseraugst)
Ulrich Georg Zuber, Roche (Kaiseraugst)
Thank you for your attention!

Questions

LIGHTCYCLER, MAGNA PURE, MYCOTOOL and VIROTOOL are trademarks of Roche.
Doing now what patients need next
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

EDQM International Microbiology Symposium
11 October 2017

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


  - 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
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 $\geq 80^\circ C$ or drained at end of day

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

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

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

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
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

• 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

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

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 $2.6 \times 10^4$ 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