New Rapid Microbiological Techniques for the Detection of *Legionella*

Elise Maynard
Agenda

- Background
- ISO 17025 & ISO 11731
- VBNC
- New techniques
  - PCR, IMS, ATP, MALDI-ToF, MPN
- Lab v Point of Care testing
Background

• HSE requirement for “evidence-base”
• Consumer demand
• Independent liaison group
  • HSE, PHE, WMSoc, LCA
• Strengths and weaknesses of techniques
Aims & Objectives

• Increase awareness of scientific techniques
  • Review verification & validation data
  • Provide publications in “Waterline”
  • Arrange events to communicate findings
  • Create factsheets to guide membership
ISO 17025:2017

• General requirements for the competence of testing and calibration laboratories
  • Adds a definition of “laboratory”
  • Applies risk-based thinking
  • Introduces greater flexibility for processes etc.
ISO 11731:2017

• Water quality – Enumeration of *Legionella* spp
  • Selection on water type and bacterial count
  • Matrix of testing procedures
    • Concentration step
  • Variable sensitivity
VBNC

• Viable But Non-Culturable
  • Very low metabolic activity
  • Do not divide, **but** are alive
  • Are able to become culturable once resuscitated
MALDI-ToF

- Matrix Assisted Laser Desorption Ionisation Time of Flight
  - Most *Legionella* spp
  - Requires ISO 11731 culture so only detects live cells
  - Highly specific, useful for confirmation tests
  - Capital equipment outlay
MALDI-ToF

Image courtesy of K Murray
qPCR

- Polymerase Chain Reaction
  - Lp sg1 & spp
  - Replication of DNA
  - Requires concentration/extraction steps
  - ISO TS 12821:2012
  - Capital equipment outlay
PCR

1. Denaturation at 94-96°C
2. Annealing at ~68°C
3. Elongation at ca. 72 °C
Increased use of qPCR assay:

- Results in 2 days i.e. time savings
- Use as a negative screening tool
  - Rule out outbreak sources
  - Use to monitor following remedial actions, such as cleaning and disinfection

http://www.hse.gov.uk/legionnaires/faqs.htm
HSE FAQ

• Tool for routine monitoring of *Legionella* trends
  – Interpretation by a competent person
  – Live & dead cells
  – GU not directly comparable with action and alert levels for CFU in HSG274

http://www.hse.gov.uk/legionnaires/faqs.htm
MPN

- Most Probable Number
  - Lp sg1
  - No concentration/ Colour change technique
  - Can be quantitative, algorithm for CFU
  - Detects live cells
  - Relatively little equipment outlay
MPN
IMS

- Immuno-Magnetic Separation
  - *Legionella* spp
  - Requires concentration, uses magnets
  - Can be quantitative with photometer
  - Detects live cells
  - Relatively little equipment required
ATP

• Adenosine Tri-Phosphate
  – Detects metabolic activity
  – Doesn’t require a concentration step
  – Only detects live cells
  – Limit of detection variable
  – Little equipment outlay
ATP
Lateral Flow

• Antibody/Antigen
  – Lp sg1
  – Requires a concentration step to increase LoD
  – Detection of live & dead cells
    • May be dependent on disinfection method
  – Little equipment outlay
Lateral Flow
Laboratory v Point of Care

• Why test?
  – WSP/Compliance/Trend analysis/monitoring
  – Outbreaks

• Where to test?
  – Compliance
  – Equipment outlay
Training

• Manufacturer
  – Instructions for use
  – You-Tube
  – Bespoke
Training

• Sampling standards
  – ISO 5667/19458
  – BS 6068/7592/8554
  – Aseptic technique

• Test standards
  – ISO 17025, ISO 11731, ISO 16266
Quality Control

• Positive/negative controls
• Records
  – Batch no
  – Use by date
  – Manufacturer QC
Cradle to Grave Sampling to Test Reports

SCI, LONDON SW1X 8PS

22nd March 2018
<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
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<tr>
<td>09:30 – 09:50</td>
<td>Registration and Refreshments</td>
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<tr>
<td>09:50 – 10:00</td>
<td>Welcome by Chairman</td>
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<tr>
<td>10:00 – 10:30</td>
<td>“Which Standard should I Follow?”&lt;br&gt;A brief overview of ISO standards 5667-1 &amp; 19458&lt;br&gt;Where should I use BS 7592, 8554 &amp; 8558?</td>
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<td>10:30 – 11:00</td>
<td>“UKAS Accredited Laboratory Test Reports”&lt;br&gt;ISO/IEC 17025</td>
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<td>11:00 – 11:30</td>
<td>Coffee break</td>
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<td>11:30 – 12:00</td>
<td>“Legionella Sample Size Reporting Requirements”&lt;br&gt;Jennifer Newton, Express Micro Science&lt;br&gt;ISO 11731:2016</td>
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<td>12:00 – 12:30</td>
<td>“New Testing Technologies”&lt;br&gt;Advantages</td>
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<td>12:30 – 13:00</td>
<td>Roundtable Q&amp;A - Questions to the panel from the delegates</td>
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<td>13:00 – 14:00</td>
<td>Lunch</td>
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<td>14:00 – 14:30</td>
<td>“WMSoc Factsheets”&lt;br&gt;Elise Maynard&lt;br&gt;qPCR</td>
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<td>14:30 – 15:00</td>
<td>“In-house Water Testing – Real-life Experiences”&lt;br&gt;Dr Paul McDermott, PJM-HS Consulting Ltd.&lt;br&gt;Why sample and test?</td>
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<td>How to test effectively?</td>
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<td>15:00 – 15:30</td>
<td>“HSE – Testing &amp; Monitoring Legionella?”&lt;br&gt;Who can be appointed?</td>
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<td>How do I interpret results?</td>
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<td>15:30 – 16:00</td>
<td>Roundtable Q&amp;A - Questions to the panel from the delegates</td>
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<tr>
<td>16:00 – 16:15</td>
<td>Summary, Close and Refreshments</td>
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