Development of rapid assays for the presence of *Legionella*, *Mycobacteria* and *Pseudomonas* spp. in hospital water systems

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HFS Clydebank
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Background

Waterborne pathogens account for a portion of HCAI each year

New guidance being developed for water safety in healthcare

Gold-standard is traditional plate culture for detecting waterborne pathogens

Reporting of results can be a lengthy process (2 and 28 days)

Delays in notification can impact on patient care

Microorganisms of particular interest are

- *Legionella* species - culture time 14 days
- Environmental *Mycobacterium* (culture time up to 6 weeks)
- *Pseudomonas aeruginosa*

Real-time PCR assays are being developed to detect & enumerate multiple waterborne pathogens to enable reporting of results on the same day.
New Suite of Guidance

HTM 0101 - Management and decontamination of surgical instruments (medical devices) used in acute care.

HTM 0106 - Decontamination of flexible endoscopes

HSG274 Part 4 - The control of Legionella and other infectious agents in Spa Pool Systems

PHE guidance for Health Protection Teams on

• Investigation of legionella cases, clusters and outbreaks
• Investigation of household water systems in cases of Legionnaires’ disease
• Responding to the detection of legionella in healthcare premises
• Guidance on the control and prevention of Legionnaires’ disease in England Technical paper 1 – disease surveillance

HTM 0401 - Safe water in healthcare premises (20th MAY 2016)
Main Changes to HTM 04 01

Provide comprehensive guidance on measures to control waterborne pathogens such as *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Mycobacteria* as well as *Legionella* (not an exhaustive list)

Align with the Health and Safety Executive’s (HSE’s) recently revised Approved Code of Practice for *Legionella* (L8) and the series of HSG274 guidance documents and with Devolved Nations

Updated guidance on the remit and aims of the WSG and WSP.

Advice:

- Sampling and testing for, *Pseudomonas aeruginosa* samples now included in Part B to complement similar guidance on *Legionella*. 
Outbreaks associated with endoscopes

Wide variety of organisms found to be involved in decontamination lapses.

Only three articles detailing viral transmission (HBV and HCV) (none from the last 10 years).

Majority of cross-transmission in the literature involves bacteria,

- *Pseudomonas aeruginosa* (38/133, 29%)
- *Mycobacterium* spp. (30/133, 23%)
- *Klebsiella pneumoniae* (14/133, 11%)
- *Salmonella* spp. (7/133, 5%)
- *Serratia marcescens* (6/133, 5%)
Outbreaks associated with endoscopes

Auto Cleaning – EWD contaminated:
Outbreak of *P. aeruginosa infections* following thoracic surgeries occurring via the contamination of old bronchoscopes and AER.
*Shimono N et al. J I C. 2008 14:418-23. (7 patients in 2 months)*

Disinfection– Rinse water:
Cluster of pseudoinfections with *Burkholderia cepacia* associated with a contaminated washer-disinfector in a bronchoscopy unit.

Inspection - Scope damage:

Procedures:
Outbreak of cystoscopy related infections with *P. aeruginosa*: New Mexico, 2007.
Multidrug-resistant *Klebsiella pneumoniae* outbreak after endoscopic retrograde cholangiopancreatography (ERCP).


- 16 patients over 9 months infected
- Eventually epidemic strain isolated from one duodenoscope
- Audit indicated inadequate manual cleaning and drying prior to storage
CHRISTINE MAI-DUC, Chad Terhune
February 20, 2015, 2:58 p.m.

Los Angeles County health officials are attempting to dispel the public’s fears surrounding a deadly outbreak of antibiotic-resistant bacteria at Ronald Reagan UCLA Medical Center, saying the episode is “not a threat to the public health at large.”

The hospital disclosed Wednesday that a superbug tied to tainted medical scopes had infected seven people and exposed 179 others.

The infections—fever, chills and severe sepsis, which the scopes, said Dr. Zachary Rubin, preventable—and were linked to a medical center. Two of those people later died.
<table>
<thead>
<tr>
<th></th>
<th>EN 15883-4</th>
<th>CFPP 01-06</th>
<th>WHTM 01-06 part D</th>
<th>HTM 2030</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total viable count</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency of test</td>
<td>Weekly until established that water supply is consistently within spec and at more extended intervals after.</td>
<td>Weekly</td>
<td>Weekly</td>
<td>Weekly</td>
</tr>
<tr>
<td>Incubation temp</td>
<td>28 – 32°C</td>
<td>28 – 32°C</td>
<td>28 – 32°C</td>
<td>35±2°C</td>
</tr>
<tr>
<td>Incubation period</td>
<td>5 days</td>
<td>Examine and report after 48 hours if positive, final report after 5 days.</td>
<td>Examine and report after 48 hours if positive, final report after 5 days.</td>
<td>72 hours</td>
</tr>
<tr>
<td>Culture media</td>
<td>R2A</td>
<td>R2A, TSA or YEA</td>
<td>R2A, TSA or YEA</td>
<td>TSA</td>
</tr>
<tr>
<td>Volume sampled</td>
<td>100 ml in duplicate</td>
<td>100 ml in duplicate</td>
<td>100 ml in duplicate</td>
<td>100 ml in duplicate</td>
</tr>
<tr>
<td>Sample transport</td>
<td>Process within 4 hours or transport at 2-5°C and process within 48 hours</td>
<td>Process within 4 hours or transport at 2-5°C and process within 24 hours</td>
<td>Process within 4 hours or transport at 2-5°C and process within 48 hours</td>
<td>Process within 4 hours or transport at 2-5°C and process within 48 hours</td>
</tr>
<tr>
<td>Acceptable limit</td>
<td>&lt;10 cfu/100ml</td>
<td>&lt;10 cfu/100ml</td>
<td>&lt;10 cfu/100ml</td>
<td>0 cfu/100ml</td>
</tr>
<tr>
<td>Further advice</td>
<td>Tests for other organisms of clinical significance may need to be performed</td>
<td>Suggests trend analysis. Identification is advised if &gt;10 cfu/ml detected. Risk assessment process in place for positive samples (traffic light system)</td>
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<td>Examine filters daily and record number of cfu.</td>
</tr>
</tbody>
</table>

**Development of rapid assays for hospital water systems**
CFPP 01-06 and WHTM 01-06 use a “traffic light” system (shown below) to indicate satisfactory and acceptable levels of contamination. (Refer to the Willis publication/PHE guidance)

<table>
<thead>
<tr>
<th>Aerobic colony count in 100 mL</th>
<th>Interpretation/action</th>
<th>Colour grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 1</td>
<td>Satisfactory</td>
<td>Green</td>
</tr>
<tr>
<td>1–9 on a regular basis</td>
<td>Acceptable – indicates that bacterial numbers are under a reasonable level of control</td>
<td>Yellow</td>
</tr>
<tr>
<td>10–100</td>
<td>Risk assessment required to investigate potential problems and super-chlorinate or repeat EWD self-disinfect</td>
<td>Orange</td>
</tr>
<tr>
<td>Over 100</td>
<td>Risk assessment required to consider taking EWD out of service until water quality improved</td>
<td>Red</td>
</tr>
</tbody>
</table>

Table 2 Total viable count results guide
Identification of *Pseudomonas* spp

If a bacterial count above 10 cfu/100 mL is obtained from test water, identification of the species is advised. If a significant proportion of the microbes appear the same species from their colonial morphology, carry out an oxidase test to presumptively identify *Pseudomonas* spp. Then if the test is positive, further investigations are required to determine whether *Pseudomonas aeruginosa* is present.

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<tr>
<td><strong>Environmental mycobacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Frequency of test</strong></td>
<td>Quarterly</td>
<td>Quarterly</td>
<td>Quarterly</td>
<td>Annual</td>
</tr>
<tr>
<td><strong>Incubation temp</strong></td>
<td>28 – 32°C</td>
<td>28 – 32°C in list of requirements 35±2°C in description of method</td>
<td>28 – 32°C</td>
<td>30±2°C in list of requirements 35±2°C in description of method</td>
</tr>
<tr>
<td><strong>Incubation period</strong></td>
<td>28 days</td>
<td>28 days</td>
<td>28 days</td>
<td>28 days</td>
</tr>
<tr>
<td><strong>Culture media</strong></td>
<td>Middlebrook 7H10</td>
<td>Middlebrook 7H10</td>
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<td><strong>Further advice</strong></td>
<td>If growth is observed, identification should be carried out by specialist laboratory</td>
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Non-tuberculous mycobacteria

• Mycobacteria other than mammalian tubercle bacilli
• Ubiquitous in the environment
• >120 different species
• Classified as rapidly growing (up to 7 days) or slow growing (> 7 days)
• Source of infections include soil, water and food stuffs
• Majority of human infections probably linked to water
• Most common clinical respiratory isolates in UK are *M. avium* complex, *M. gordonae*, *M. xenopi*
• Severely underreported
Five-Year Outbreak of Community- and Hospital-Acquired *Mycobacterium porcinum* Infections Related to Public Water Supplies

Barbara A. Brown-Elliott,1 Richard J. Wallace, Jr.,1* Carmen Tichindelean,2‡ Juan C. Sarria,2
Steven McNulty,1 Ravikaran Vasireddy,1 Linda Bridge,1 C. Glenn Mayhall,2
Christine Turenne,4 and Michael Loeffelholz3

Hospital’s water system implicated in *Mycobacterium gordonae* pseudo-outbreak

**V. Moore, K. Brooks, S. Dauenhauer, L. Jett, R. Washburn**
Overton Brooks VA Medical Center, Shreveport, Louisiana
Abstract ID 50687 Monday, June 20

**An Outbreak of Bacteremias Associated With *Mycobacterium mucogenicum* in a Hospital Water Supply**

Susan Klinea1a2 c1, Sarah Camerona1, Andrew Streifela1a3, Mitchell A. Yakrusa4, Frank Kairisa5, Keith Peacocka6, John Bessera7 and Robert C. Cookseya4

**Nontuberculous Mycobacteria from Household Plumbing of Patients with Nontuberculous Mycobacteria Disease**

Joseph O. Falkingham, III
Customer phoned for advice

Final rinse water ACCs varying between 0 and >100 regularly

Some machines failing one week, others the next

Some distrust of lab results

Monitoring of trends in results over time showed more consistent pattern
Development of rapid assays for hospital water systems

Machines 2 & 4

Machine 3 Typical pattern

Courtesy of Malcolm Greenhalgh
Mains supply

Softener

Filters? / Storage tanks?

RO Machine (supply water)

Washer Disinfector (final rinse water)
Following Up Poor Final Rinse Results

Self-disinfect cycles being run regularly?
Filters changed regularly and maintained appropriately?
RO membrane maintained and changed at correct intervals?
Softener filter changed regularly?
Appropriate sampling points?

AIM TO PREVENT BIOFILM FORMATION
Legionnaires’ disease

Figure 1. Notification rate of Legionnaires’ disease in the EU/EEA* by year of reporting, 1995–2014

from 2003-2014
Developed and validated PCR assay to detect and quantify *Legionella* spp, *L. pneumophila*, *L. pn* sg-1, *Mycobacteria* spp and *Pseudomonas aeruginosa* in a range of environmental samples

Used on limited public health basis from Porton lab following agreement between LA, HPU and FW&E

Results for urgent samples within 24 hours of sample receipt
What happens with samples?

- Water sample filtered (100ml – 1L)
  - Standard culture
    - Results in 4 – 42 days
  - DNA extraction
    - PCR
    - Results in ~24 hours
How does it work?

Development of rapid assays for hospital water systems

Detecting the molecular fingerprint

Results in real-time

Approximately 2 hours

Results expressed as genome units (GU) / volume
Why do we need PCR testing?

‘Gold standard’ culture is slow
Other flora interfere
Cannot detect VBNC cells
Improved sensitivity
Faster public health responses
Need to move with the times!

Development of rapid assays for hospital water systems
Benefits of PCR testing

- Can detect all known species (*Legionella* spp., *Mycobacteria* spp., *Ps* spp.

- Can distinguish
  - Legionella serogroups 1, 2-14 and species
  - *M. avium, M. chimera* – no need for further confirmations

- Quantifiable and fast

- Sensitive, specific and reproducible

- Background flora typically do not interfere

- Provides more information about the water system

- **Can type strains direct from environmental DNA where one strain predominates**

- **Appreciable public health benefits**
How do we interpret results?

- Not appropriate to directly compare culture to PCR
- PCR will detect dead, dying or injured cells
- Cells inside amoeba
- VBNC cells
- Species that simply will not grow in the laboratory
- All need to be considered when interpreting results.
- Usually means a PCR result is higher than corresponding culture
How do we interpret results?

- Hindered by current regulatory guidance – PHE working actively with HSE to derive appropriate guidelines for use of PCR

- Detection of low levels of DNA good indicator of low risk

- **Negative predictive value** NPV = 98.3% for PHE samples (2500 samples)

- Currently used for public health incidents to rapidly rule out potential sources

- Could have a culture negative, PCR positive sample. How do we interpret?
How do we interpret results?

- Detection of Legionella or Mycobacteria, whether dead or alive indicates a recent contamination that should be investigated further
  - Evidence that free DNA is quickly removed by systems with good turnover of water – French National guidelines.

- Provides a fast indication of potential sources in outbreaks

- Numbers (GU/L) aren’t necessarily important in PH investigations
Key message

- The usefulness of the result depends on the quality and timeliness of the sample.
- Results must be interpreted in the context of the sample e.g. where was it taken, how, what was the biocide treatment, temperature, history of the H&CWS/AER, % positives?
- Failure to obtain this information can make interpretation difficult.
When to use PCR

- Public health investigations/outbreaks – rapid identification of potential sources, rapidly rule out sources
- When culture is expected to be difficult
- When you think you have a problem
- Following remedial action – rapid answer ‘is the system safe?’ Remove that “out of service” sign
- Risk management – monitoring high risk or out of control H&CWS or AER.
- Routine monitoring – making progress
Directly linking patients to sources

Direct, nested sequence based typing (7 PCRs (in duplicate) followed by sequencing of all the PCR products)

Produces a series of 7 numbers which is given a unique sequence type identifier e.g. ST1, ST47, ST62 etc.

Can be performed directly on environmental DNA extracts and patient samples without having to grow Legionella by culture

Can be done in <24 hours from sample receipt – but very expensive at £500/sample

Currently working with Ref lab on new clinical assay that can simultaneously diagnose and sequence type the most common strains in less than 90 minutes

Development of rapid assays for hospital water systems
Public Health benefit has been proven

Development of rapid assays for hospital water systems
Heated birthing pools as a source of Legionnaires’ disease

S. L. COLLINS¹*, B. AFSHAR¹, J. T. WALKER¹, H. AIRD¹, F. NAIK¹, F. PARRY-FORD¹, N. PHIN¹, T. G. HARRISON¹, V. J. CHALKER¹, S. SORRELL² and T. CRESSWELL³

¹ National Infection Service, Public Health England, UK
² Environmental Health Officer
³ Health Protection Team, P.

Received 10 April 2015; i

ORIGINAL ARTICLE

Real-time PCR to supplement gold-standard culture-based detection of Legionella in environmental samples

S. Collins¹,², F. Jorgensen², C. Willis² and J. Walker¹

¹ Public Health England, Biosecurity Investigation Unit, Salisbury, UK
² Public Health England, Food, Water and Environmental Microbiology Laboratory, Salisbury, UK

Development of rapid assays for hospital water systems
Going forward

Mycobacteria PCR assay now developed and undergoing trials

HTM 0106 – Decontamination of flexible endoscopes “In Press”

HSE/PHE collaboration – potential for inclusion of PCR in ACoP L8 future issues?

Water Management Society “Rapid Microbiology Methods Liaison Group” – best practice documents for end users

New PHE Legionella PCR quality assurance scheme
Culture is still currently our best tool to epidemiologically link patients to sources but PCR can provide faster results and information when culture is not possible (clinical or environmental).
Development of rapid assays for hospital water systems

- Identify specific species – may help in management of water systems
- Reduces time of culture tests by upwards of 2 days
- No presumptive results
- Very cheap (minus capital cost)

But…

- Culture dependent
- Environmental database is lagging behind clinical
Is your water really safe to use?

Culture is the Gold Standard – but suffers from a number of deficiencies

- VBNC
- Selectivity
- Not incubating mycobacteria for long enough? Limitations of culture methods.

PCR can be an alternative

- Rapid assay
- Will detect all species
- Direct sequence typing from the DNA
- Currently used for public health incidents for Legionella

Assay for *Mycobacteria spp.* and *Pseudomonas* being trialled
Thanks to
Food Water and Environmental Laboratories
Tina Bradley
Caroline Willis
Samuel Collins
Water Management Society
Health and Safety Executive
HIS Rinse Water Review Group