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

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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

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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

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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

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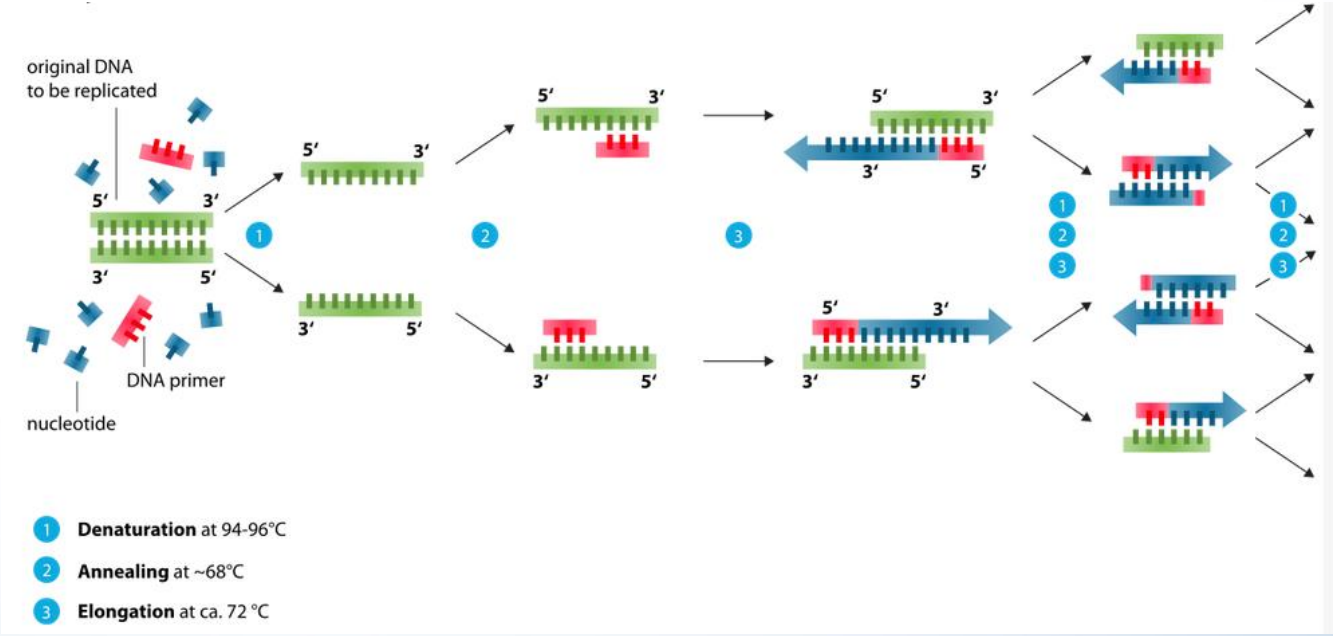
PCR



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HSE FAQ

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

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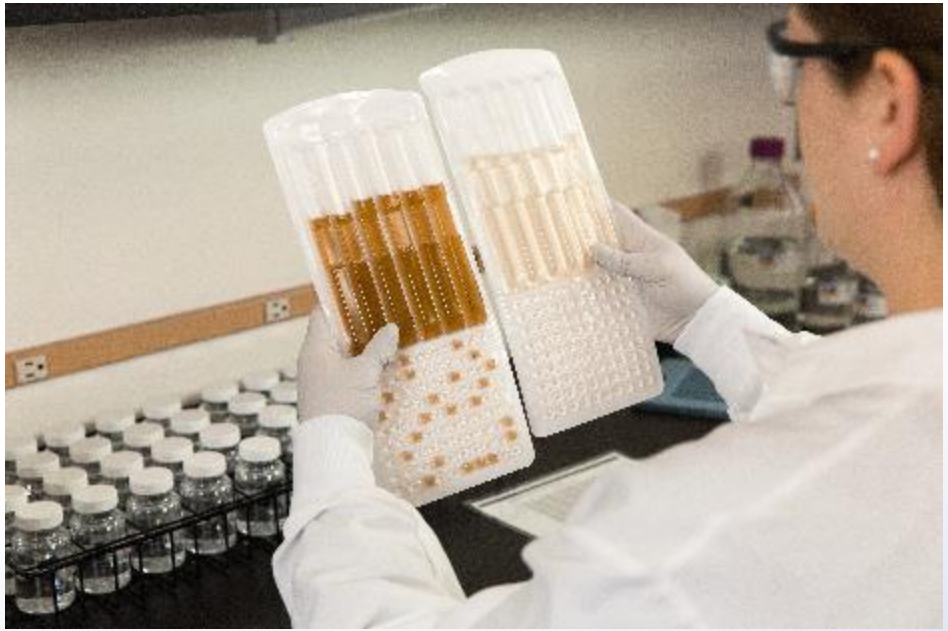
MPN



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IMS

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

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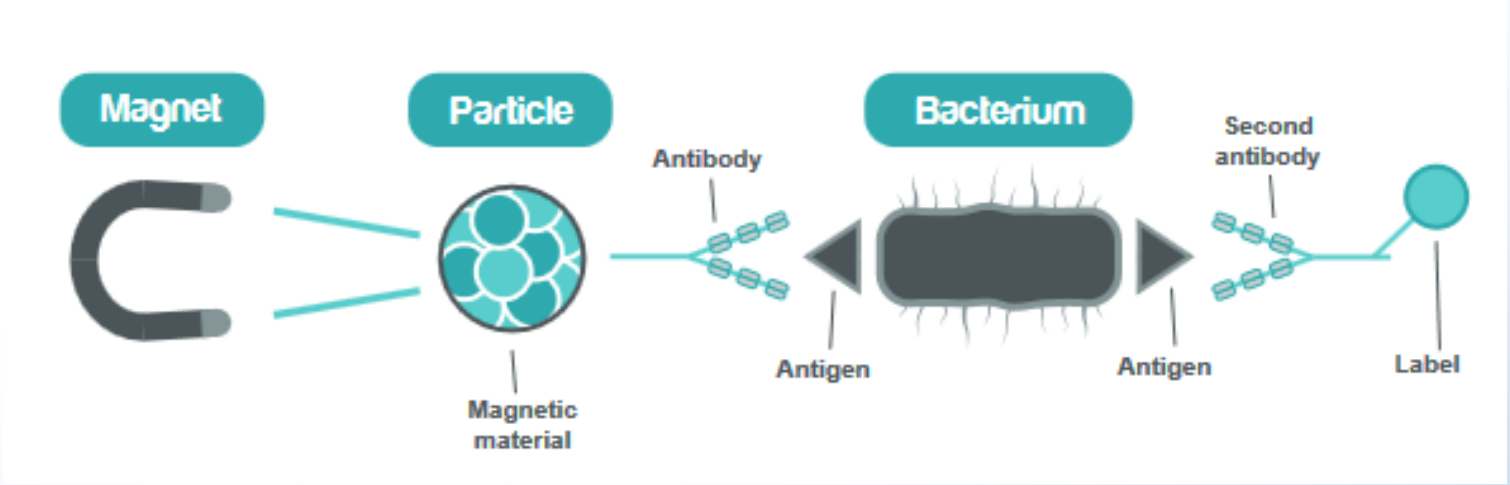
IMS

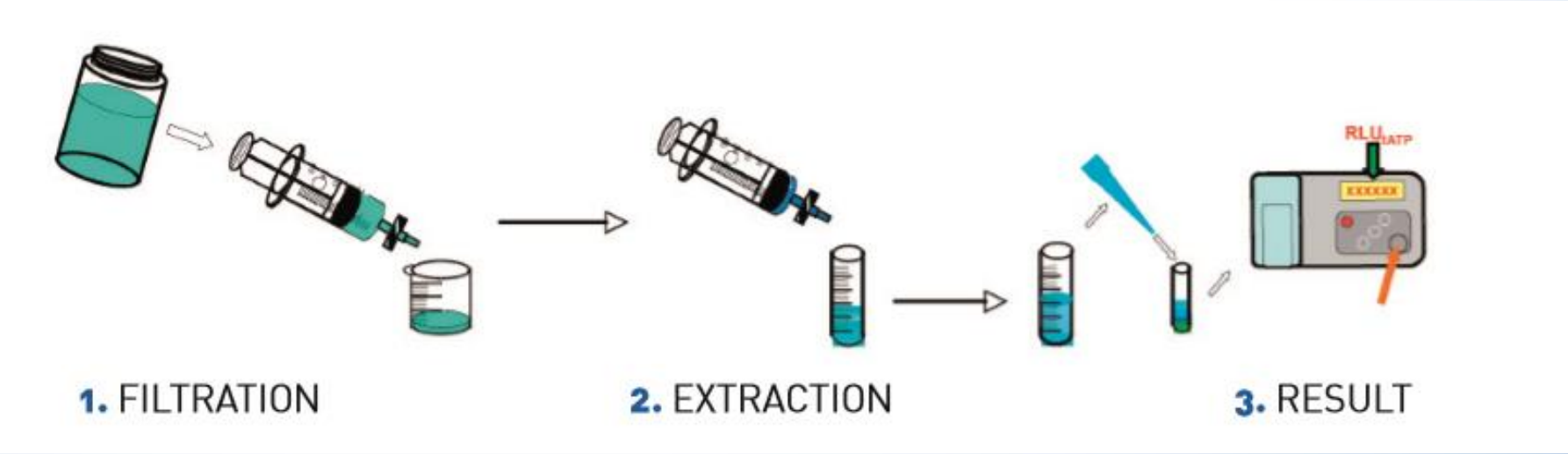

Magnet
Particle
Bacterium
Antibody
Second antibody
Magnetic material
Antigen
Antigen
Label

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



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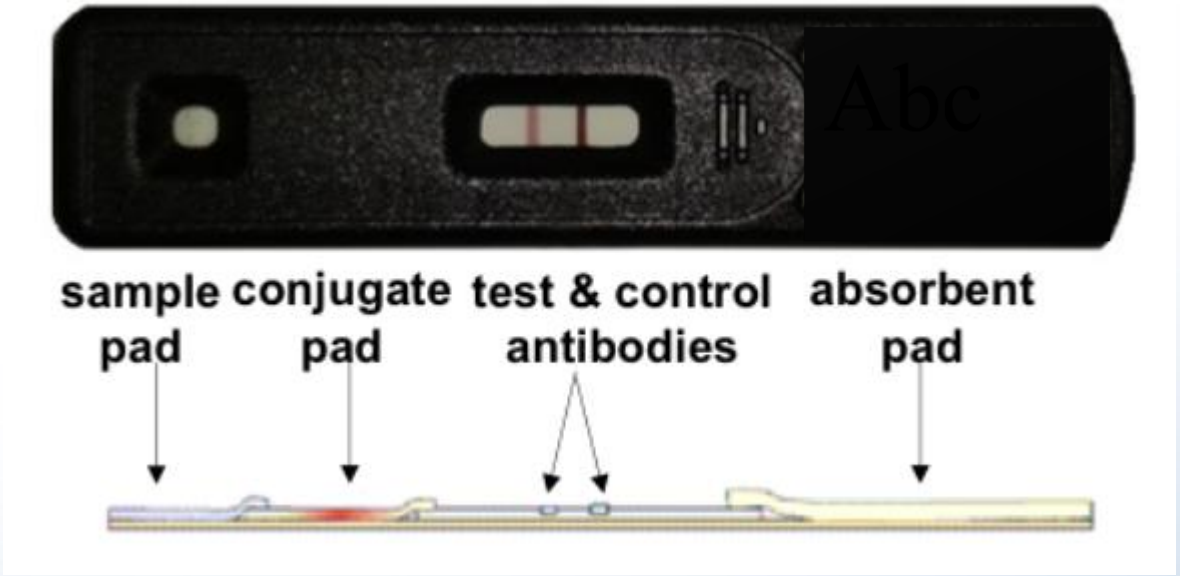
Lateral Flow

- 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

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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

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Quality Control

- Positive/negative controls
- Records
  - Batch no
  - Use by date
  - Manufacturer QC



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ONE DAY EVENT

Cradle to Grave

Sampling to Test Reports

SCI, LONDON
SW1X 8PS

22nd March 2018

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Programme

09:30 – 09:50	Registration and Refreshments
09:50 – 10:00	Welcome by Chairman
10:00 – 10:30	"Which Standard should I Follow?" *A brief overview of ISO standards 5667-1 & 19458* *Where should I use BS 7592, 8554 & 8558? | What about HTM 04-01 in Healthcare?*
10:30 – 11:00	"UKAS Accredited Laboratory Test Reports" *ISO/IEC17025 | Decoding test reports* *Detection and enumeration of Legionella – reporting under accreditation*
11:00 – 11:30	Coffee break
11:30 – 12:00	"Legionella Sample Size Reporting Requirements" Jennifer Newton, Express Micro Science *ISO 11731:2016 | Sample volumes | Limits of detection*
12:00 – 12:30	"New Testing Technologies" *Advantages | Limitations | Practical applications*
12:30 – 13:00	Roundtable Q&A - *Questions to the panel from the delegates*
13:00 – 14:00	Lunch
14:00 – 14:30	"WMSoc Factsheets" Elise Maynard *qPCR | MALDIToF | IMS*
14:30 – 15:00	"In-house Water Testing – Real-life Experiences" Dr Paul McDermott, PJM-HS Consulting Ltd. *Why sample and test? | What problems may be encountered?* *What are the benefits of in-house testing?* *How to test effectively? | What are the drawbacks?*
15:00 – 15:30	"HSE – Testing & Monitoring Legionella?" *Who can be appointed? | How do I test? | How often should I test?* *How do I interpret results? | Current HSE position on qPCR*
15:30 – 16:00	Roundtable Q&A - *Questions to the panel from the delegates*
16:00 – 16:15	Summary, Close and Refreshments

TRADE STANDS AVAILABLE

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