Government of Western Australia
PathWest

PathWest
LABORATORY MEDICINE WA

Understanding the Microbiological Requirements of AS 4187:2014 Amd 2:2019

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Disclosures

- Employed by PathWest Laboratory Medicine, Government Organisation, recently became our own Health Service Provider.
- WA's Public Health Laboratory, Reference Laboratories (Mycobacteria, Enteric, Molecular Epidemiology).
- 5 environmental laboratories, test WA's drinking water and AFERs.
- Advise on secondary and tertiary hospital infection control matters and water quality risk assessment.
- No conflicts

I'm calling from the endoscopy clinic. The lab has just told us we have 2 colonies of *Pseudomonas* in our Soluscope!

What should we do?

Yikes….

- What's a Soluscope? What does it do?
- Is *Pseudomonas* bad?
- 2 colonies…is that good?
- What are they expecting from me?
- It's their machine, shouldn't they know what to do?

Good news: Amd 2 makes the microbiology easy

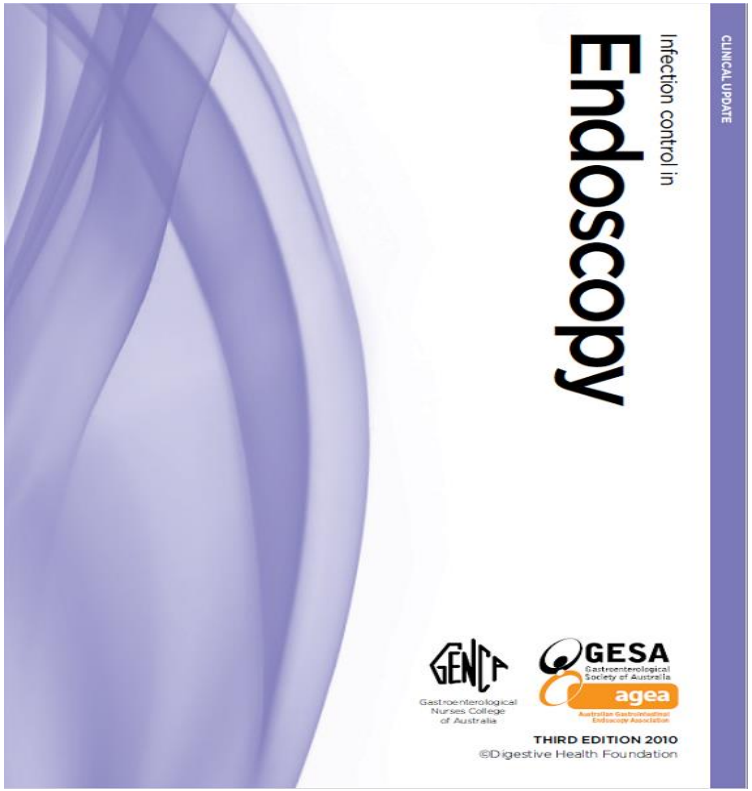
- Only 2 Tables in Section 7!

- Clear requirements for:
  - What microbiological analyses to perform
  - What laboratory methods to use
  - When and how often to test
  - What is a pass or fail

- But not how to remediate an out-of-specification result…

What AS 4187 is not

Not a replacement for GENCA/GESA guidelines for the microbiological surveillance of endoscopes:



AFER = Automated Flexible Endoscope Reprocessor
AER = Automated Endoscope Reprocessor

Soluscope

Medivator

STERIS Reliance EPS…others

GENCA has endorsed AS 4187 Amd 2

- Monthly surveillance
- Sampling method doesn't sufficiently address different AFERs
- Sample volumes variable
- Lab procedures impractical, inappropriate media

- Interpretation less clear
- Contamination concepts, actions

- Monthly surveillance
- Sampling method more clear (however still some interpretation issues)
- Sample volume 100ml per analyte
- Lab procedures clearly defined, referenced, appropriate media for water
- Environmental lab (NATA 17025)
- Clear pass/fail

Why is the microbiology important?

- Water quality can affect the final product if it contains contaminants that are not removed prior to use – chemical, microbiological
    - Optimal equipment functioning
    - Minimise biofilm build-up in internal pipework
    - Physico-chemical induced corrosion can increase biofilm
    - Potential for high contamination of incoming water to overwhelm the onboard filtration/disinfection processes
    - Protect RMDs from being contaminated while being decontaminated

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TABLE 7.2

FINAL RINSE WATER—MANUAL CLEANING MANUAL DISINFECTION AND WASHER-DISINFECTORS

Substance	Specifications
[redacted]	
Total viable count (see Note)	≤ 100 cfu/100 mL
Endotoxin	≤ 0.25 EU/mL

NOTES:

1 Table 7.2 applies to the quality of water used in the types of washer-disinfectors included under the scopes of ISO 15883-1 and ISO 15883-2.

2 ISO 15883-1 is the umbrella (i.e. horizontal) standard that applies to all WDs. Specific or altered requirements are given in each of its subsequent parts (i.e. vertical standards) for different types of WD. See Table 7.3 for specific requirements for WDs used to reprocess thermolabile endoscopes.

3 For TVC, test methodology should be in accordance with ISO 15883-1 and the HTM 01-01 series.

TABLE 7.3

FINAL RINSE WATER—WASHER-DISINFECTORS IN ACCORDANCE WITH ISO 15883-4 FOR THERMOLABILE ENDOSCOPES

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Pseudomonas aeruginosa	Not detected/100 mL
(Atypical) *Mycobacterium sp.*	Not detected/100 mL
[redacted]	
Endotoxin	≤ 30 EU/mL

NOTE: For TVC, test methodology should be in accordance with ISO 15883-1 and the HTM 01-06 series.

ISO 15883

15883-1:2006 Washer-disinfectors – Part 1: General Requirements, terms and definitions and tests

15883-2:2006 Washer-disinfectors – Part 2: Requirements and tests for washer-disinfectors employing thermal disinfection for surgical instruments, anaesthetic equipment, bowls, dishes, receivers, utensils, glassware, etc.

15883-3:2006 Washer-disinfectors – Part 3: Requirements and tests for washer-disinfectors employing thermal disinfection for human waste containers.

15883-4:2018 Washer-disinfectors – Part 4: Requirements and tests for washer-disinfectors employing chemical disinfection for thermolabile endoscopes

Department of Health

Health Technical Memorandum 01-01: Management and decontamination of surgical instruments (medical devices) used in acute care

Part D: Washer-disinfectors

July 2016

What is a Total Viable Count?

- Total Viable Count (TVC) = Heterotrophic Plate Count (HPC) = Standard Plate Count (SPC) = Total Bacterial Count = Water Plate Count = Aerobic Mesophilic Viable Count….

- All microorganisms that use organic nutrients for growth
  - Universally present in all types of water, food, soil, vegetation, air
  - Encompasses bacteria, yeasts and moulds

- No single method will recover all organisms in the water being analysed

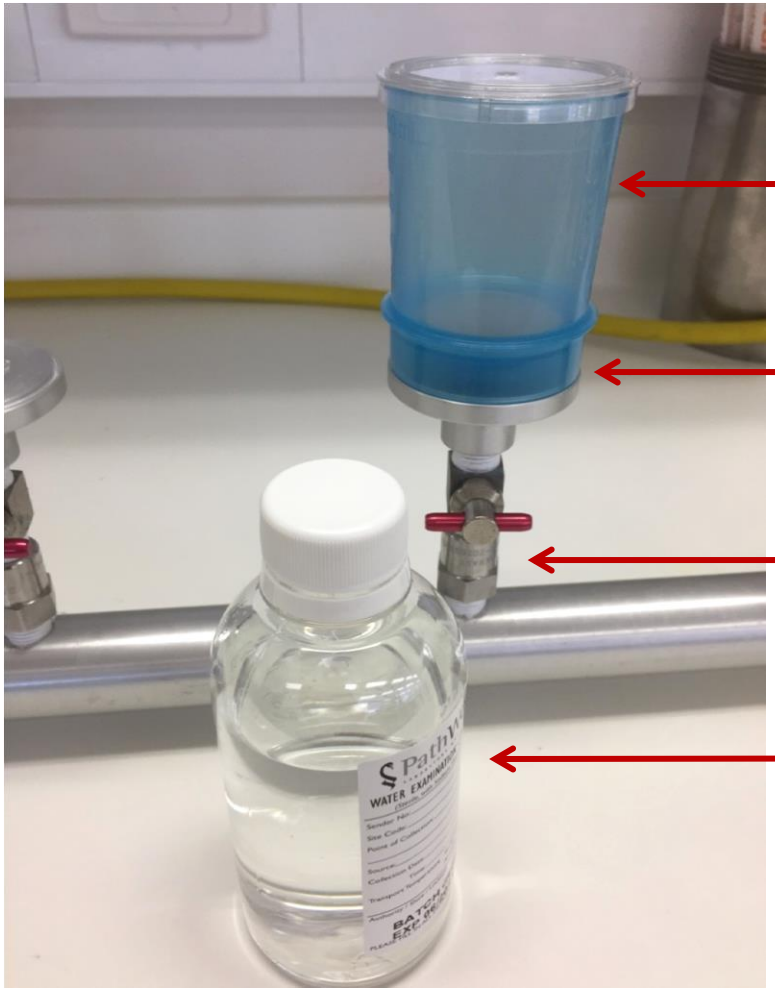
TVC Method in AS/ISO

- Sample method (**Membrane filtration**)
  - more flexible than spread/pour plate
  - any sample volume >1.0ml
  - efficient method for 100ml of water

- **R2A** – low-nutrient, low-ionic strength formulation to culture organisms that have a water-based, rather than mammalian lifestyle

- Incubation conditions (**28-32ºC for 5 days**)
  - High-temperature (35-37ºC) and short incubation time (34-48 hours) favour growth of bacteria from animals and humans
  - Low-temperature incubation (20-28ºC) and longer incubation time (5-7 days) favour growth of aquatic bacteria

Membrane filtration

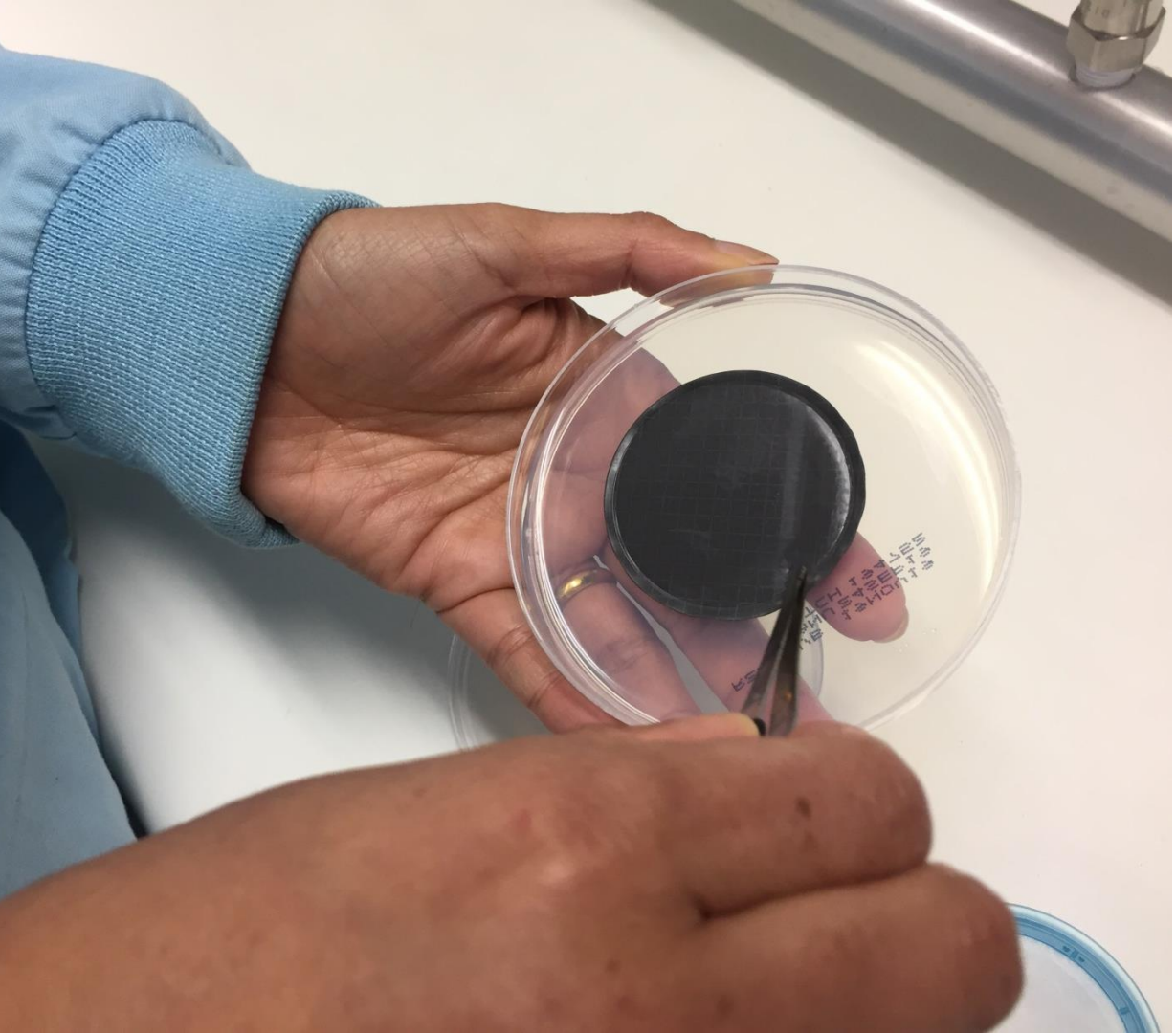


Sterile cup

Membrane filter
bottom of cup

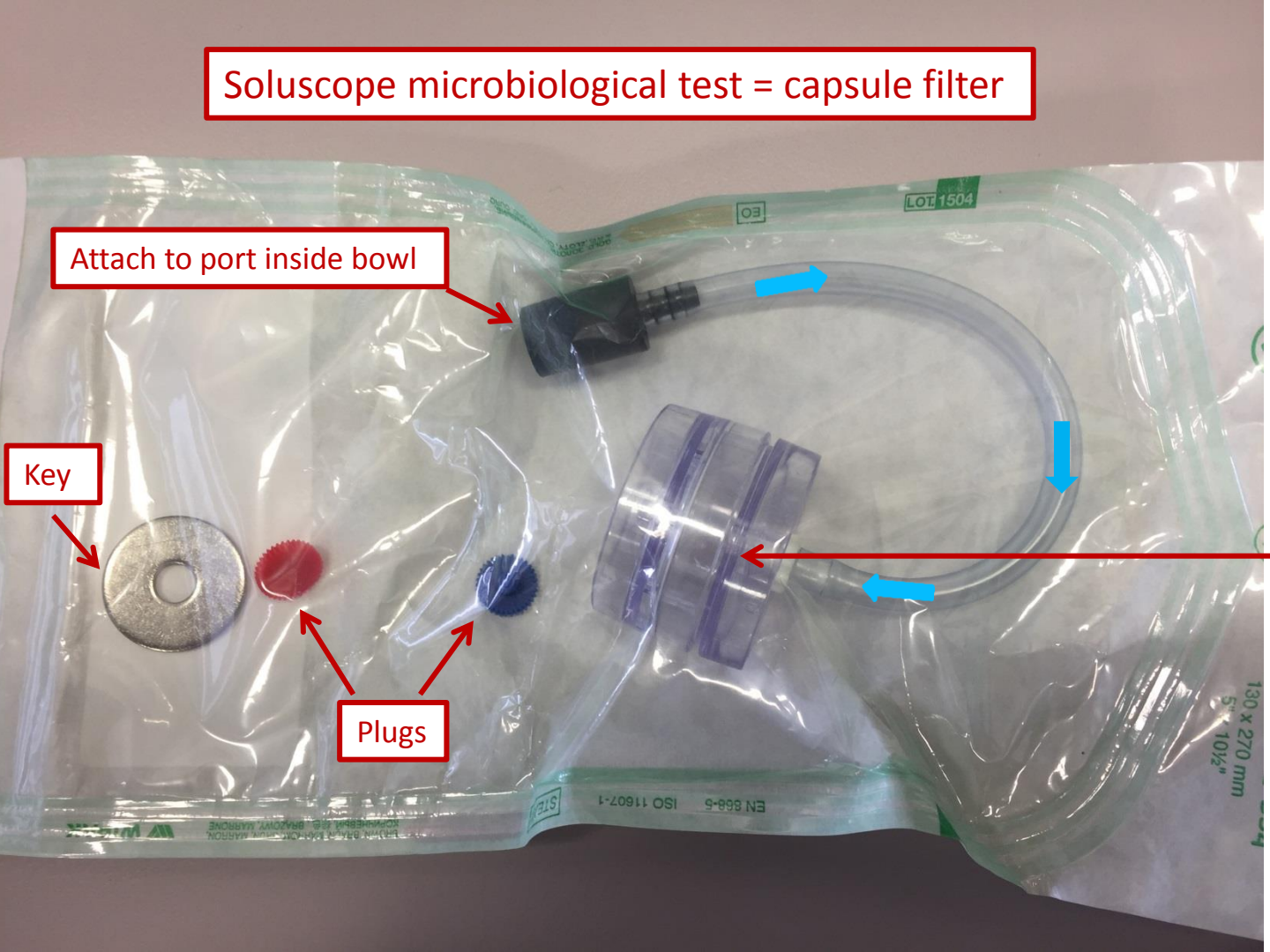
Manifold

Water sample (250 ml)

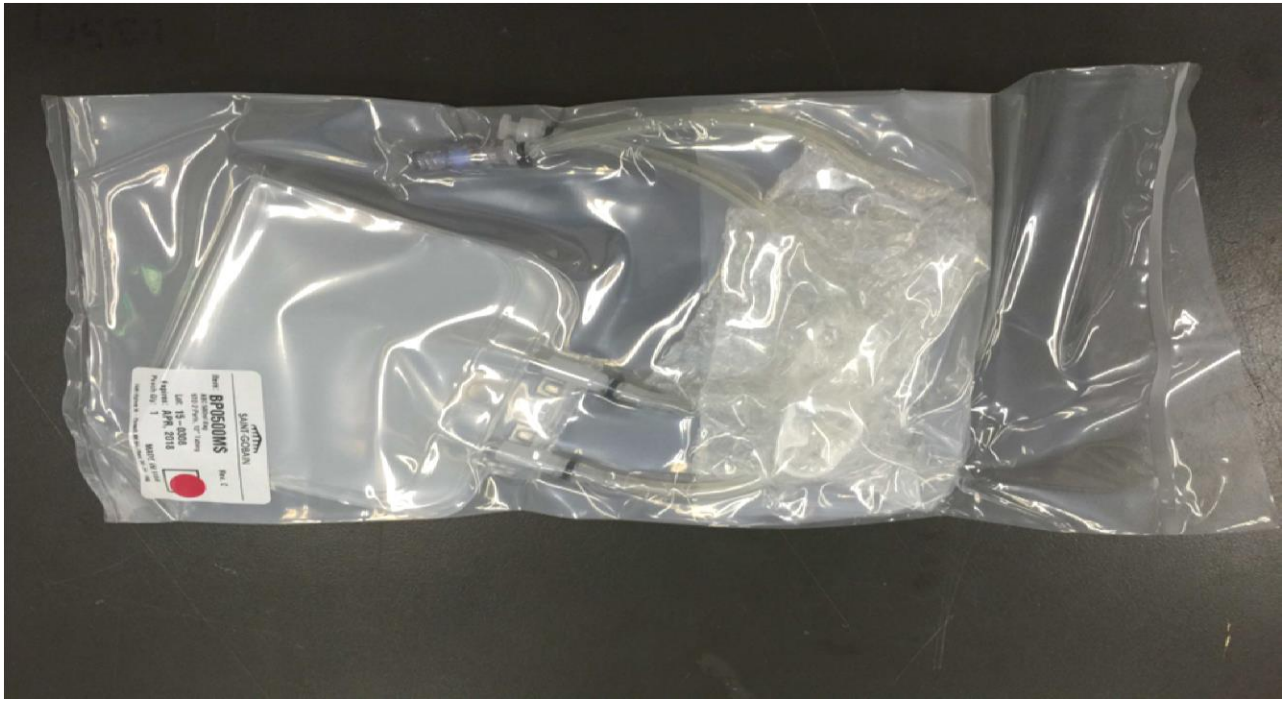


After filtration, membrane is aseptically placed on the surface of the agar plate

Soluscope microbiological test = capsule filter

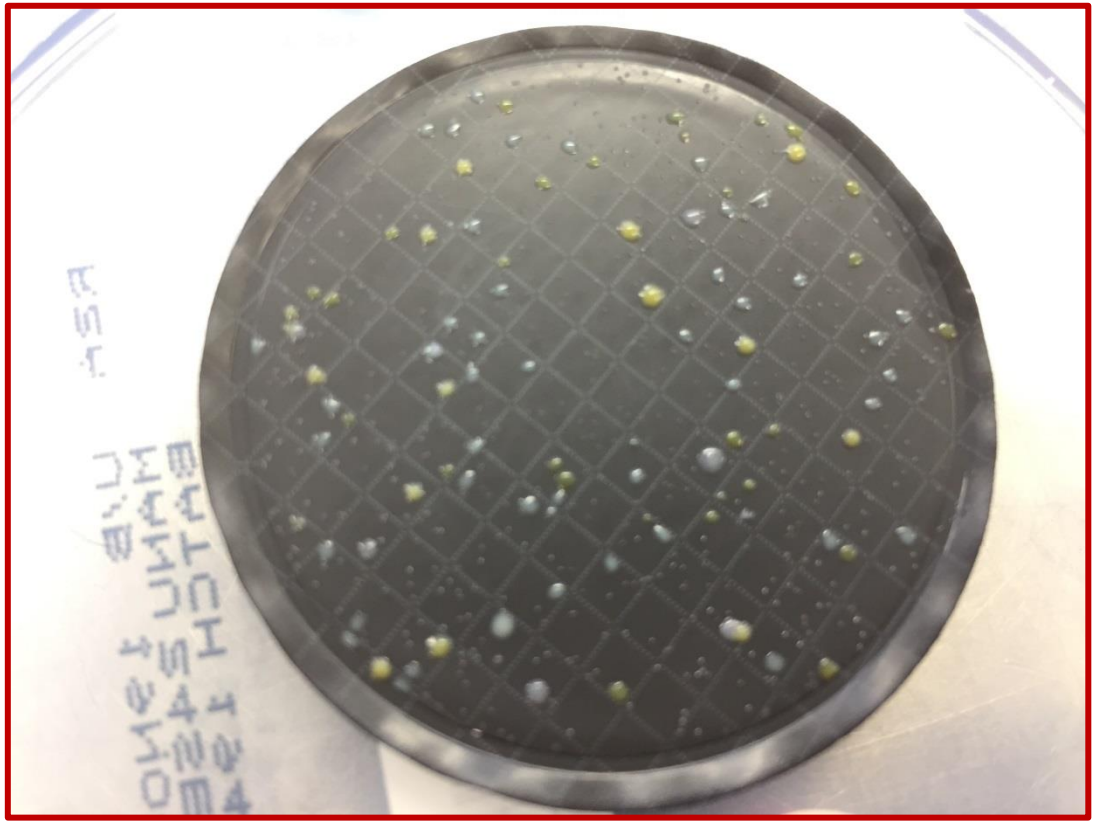


Additional sampling tips



Soluscopes

- 500ml water collection bags that fit into the bowl instead
- Pyrogen-free bags for annual endotoxin test



Mycobacteria and Legionella will **not** grow

TVC

- AFERs = final product ≤10cfu/100ml
- Batch-Washers and manual rinse water = pre-sterilisation ≤100cfu/100ml
- Not possible/necessary to differentiate which microorganisms are potentially pathogenic (except *Pseudomonas aeruginosa* for AFERs)

Will knowing the organism name/s help me?

- Skin flora? – Coagulase negative staphylococci, Micrococci, diphtheroids, Corynebacteria, Bacillus, *Staphylococcus aureus*

- High TVC with no endotoxin?
  - Maybe they are all gram positives!

- Environmental – Bacillus, fungi, mycobacteria, some Enterobacteriales, non-fermenters (Pseudomonas…)

What does AS 4187 say about my result?

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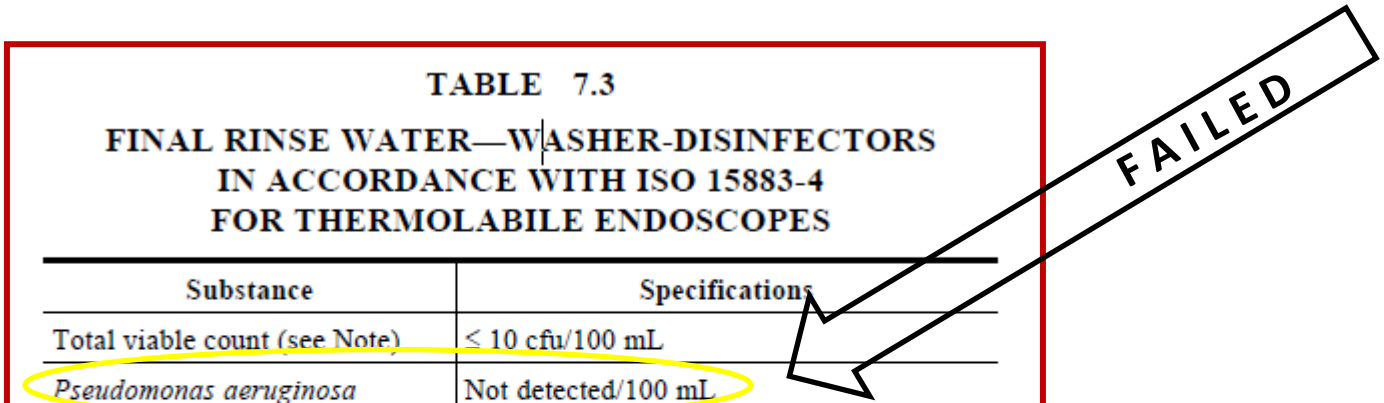


What does GENCA say about my result?

"A growth of *Pseudomonas* spp. or other nonfermentative gram-negative bacilli from a duodenoscope, bronchoscope or an AFER that processes duodenoscopes or bronchoscopes would be cause for serious and immediate concern"

What are non-fermenters and why are they important?

- Gram negatives, oxidase positive, can't catabolise glucose
- Ubiquitous in the environment, moist areas
- Biofilm formation in aerators, sinks, AFERs, catheters
- Intrinsically resistant to antibiotics and disinfectants
- Important opportunistic and nosocomial pathogens

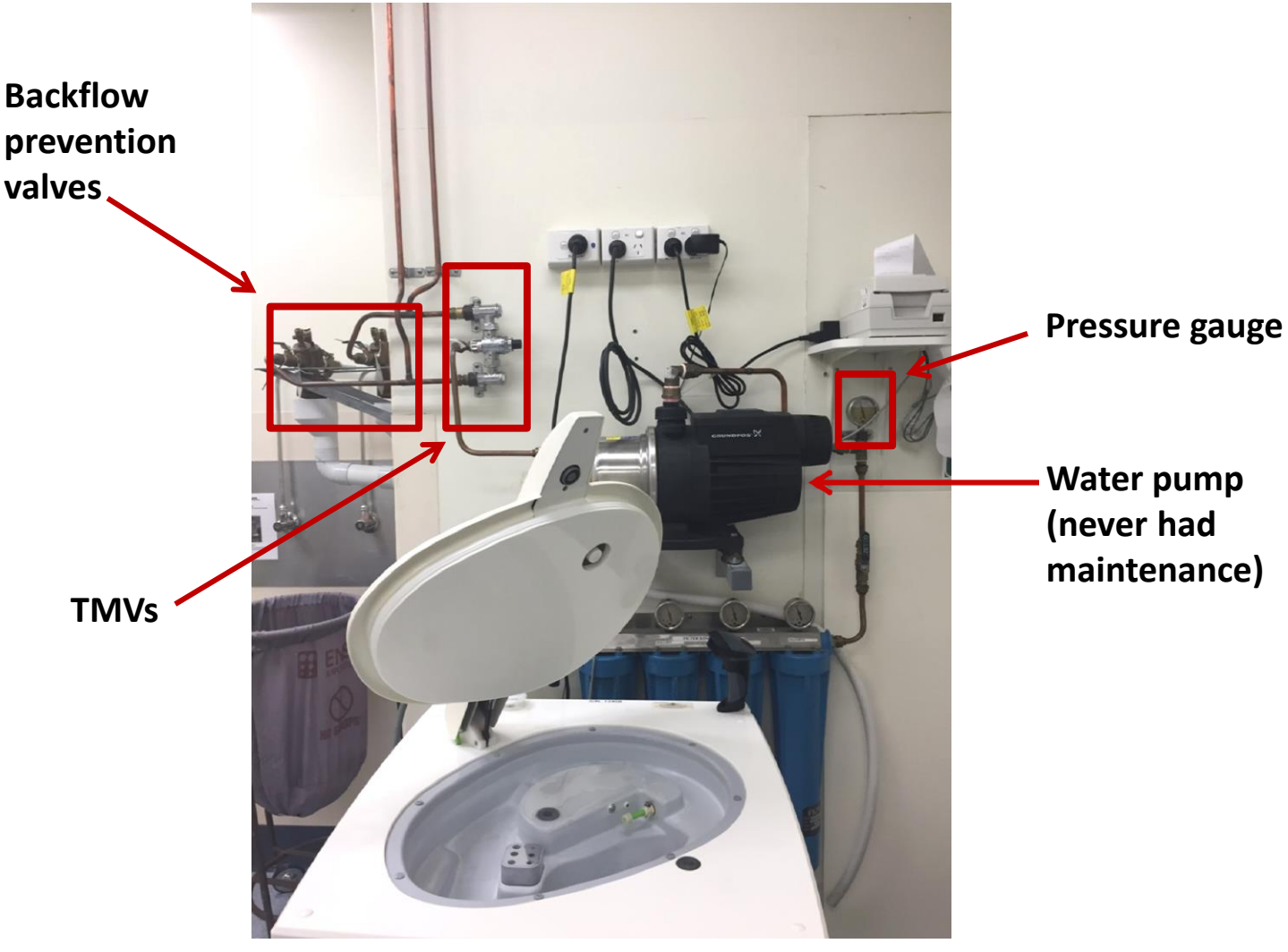
- Have differing virulence potential:
    - Higher : *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Stenotrophomonas maltophila, Burkholderia cepacia, Chryseobacterium, Shewanella…*
    - Lower: *Blastomonas, Brevundimonas, Cupriavidus…*

https://www.google.com.au/search?q=pseudomonas+biofilm+picture&tbm=isch&source=hp&sa=X&ved=2ahUKEwiN1_Spu9zlAhWUguYKHVD4DbkQsAR6BAgGEAE&biw=1920&bih=951#imgdii=UWkpCRR0kX_8aM:&imgrc=TEEKVZUDN-nl_M:&spf=1573279630790

I'm calling from the endoscopy clinic. The lab has just told us we have 2cfu/100mls of *Pseudomonas aeruginosa* in our latest Soluscope monthly surveillance sample.

What should we do?

Take the machine out of service, I'm coming for a visit.

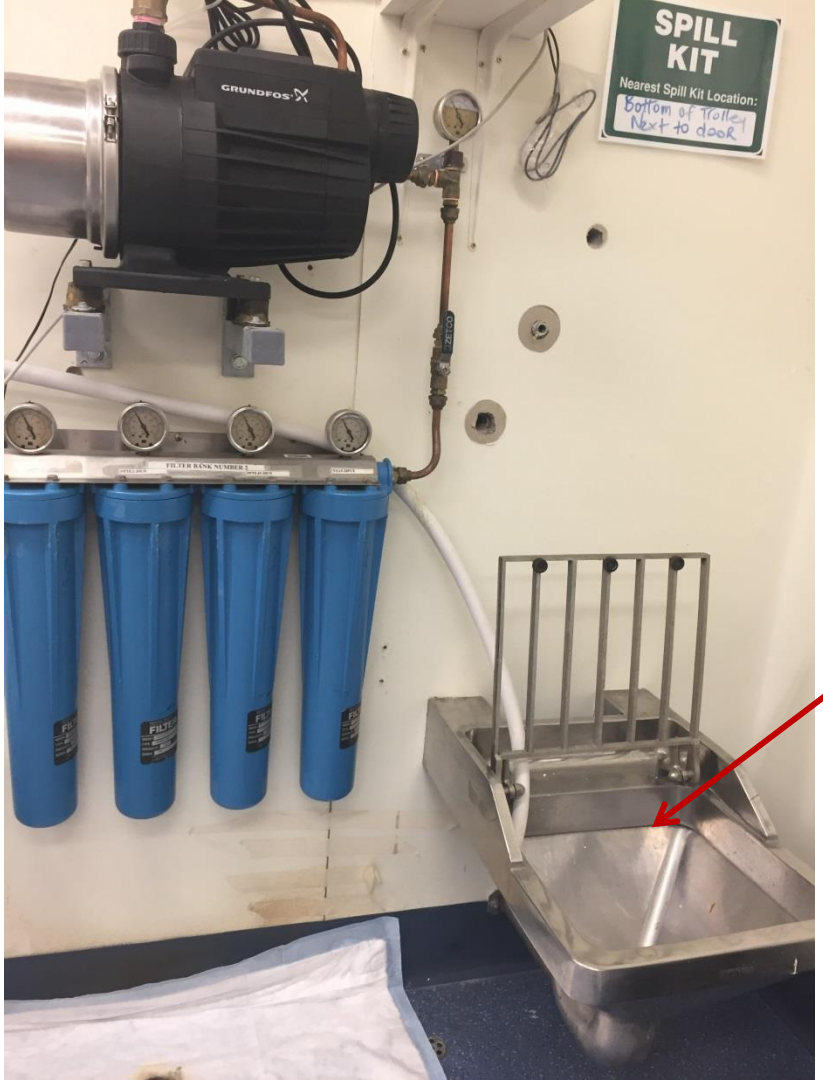

Backflow prevention valves
Pressure gauge
Water pump (never had maintenance)
TMVs

Old filter bank

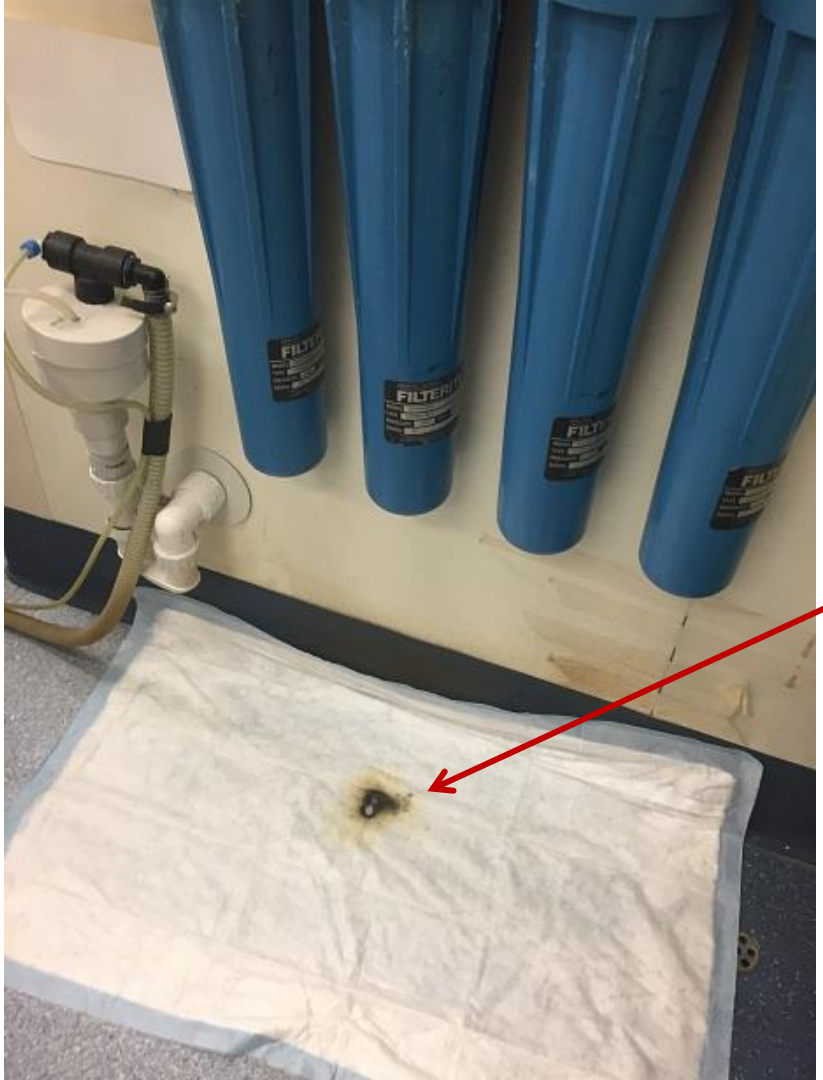
In coarse to fine sequence

Filter flushing pipe

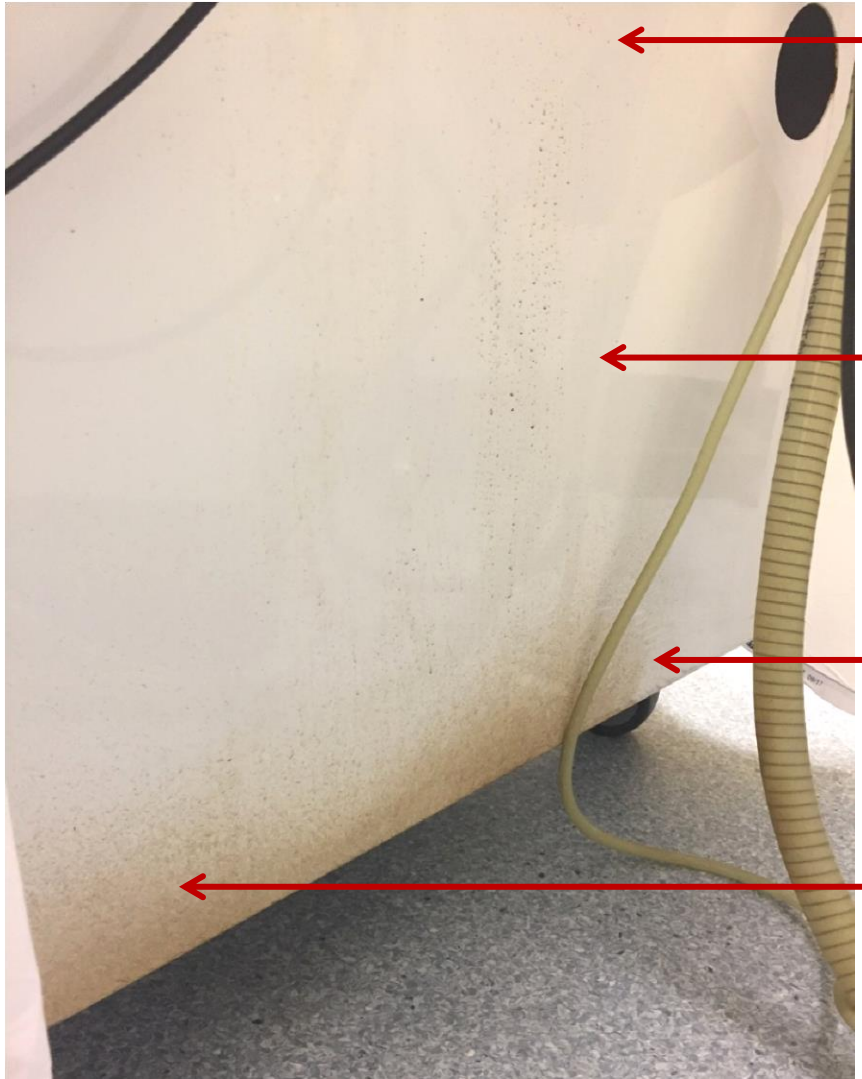
To waste where?


SPILL KIT
Nearest Spill Kit Location:
Bottom of Trolley Next to door
GRUNDFOS
FILTER BANK NUMBER 1

Old sluice



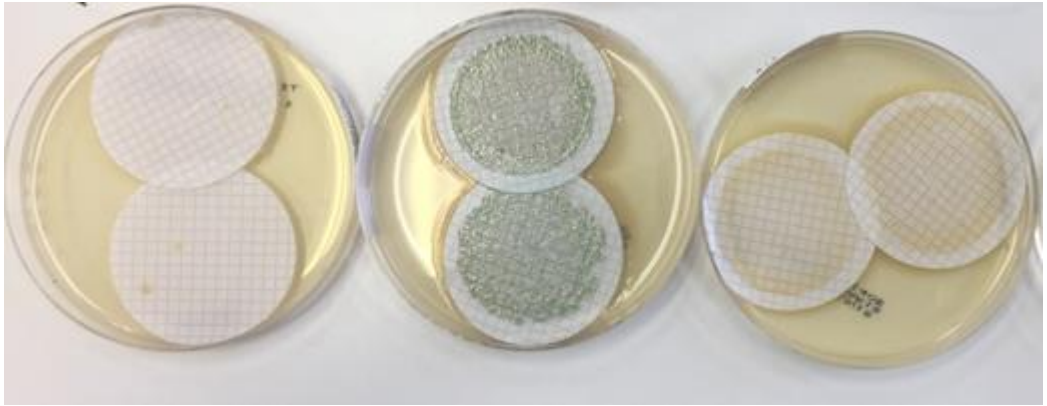

Drip from
water pump



Dripping from water pump and splashing onto Soluscope

Hunting the Pseudomonas

- Sampling back in the water supply chain:



Pre-pump **Post-pump** **Post-filter bank**

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What should we do?

Call it quits and buy a new one.

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Atypical Mycobacteria (NTM)

- 'Atypical' = Non-tuberculous *mycobacteria (*NTM*)*
- Free-living in environment, waxy cell wall, water-borne, grow distilled water

- Rapid growers (RGM) – 7 days, routine media
  - *M.fortuitum, M.chelonae, M.abscessus*

- Slow growers – up to 8-12 weeks, specialised media
  - *M.avium-intracellulare, M.gordonae, M.chimaera*

Review Article: Kovaleva et al,Transmission of Infection by Flexible Gastrointestinal Endoscopy and Bronchoscopy, *Clinical Microbiology Reviews* Apr 2013, 26(2) 231-254

Atypical Mycobacteria (NTM)

- Will *not* grow on the TVC media (7H10/11)
- ISO requires both rapid **and** slow-growing mycobacteria
- Takes 28 days for a negative result
- Need to send 200ml of water to comply (membrane filtration, required to be performed in duplicate)

- More difficult test to source
  - within the remit of highly specialised clinical microbiology labs
  - expensive

Growth of *Mycobacterium* isolated on a plate of culture medium. (Photo: Stacey Pfaller, EPA)

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Substance	Specifications
pH	5.5–8.0
Conductivity at 20°C	≤ 30 µS/cm
Total hardness	≤ 10 mg $CaCO_3$/L
Chloride	≤ 10 mg/L
Iron	≤ 0.2 mg/L
Phosphates (molybdate reactive)	≤ 0.2 mg/L
Silicates (molybdate reactive)	≤ 1.0 mg/L
Total viable count (see Note)	≤ 100 cfu/100 mL
Endotoxin	≤ 0.25 EU/mL

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What is Endotoxin?

- A type of lipopolysaccharide (LPS)
- Major component of the outer membrane of gram negative bacteria
- High doses in blood (500pg/ml) cause ‘cytokine storm’, septic shock and death

Where is Endotoxin?

- ~1gm of endotoxin in human gut
- 1 to 50 picogram/ml in plasma of healthy humans (0.01 and 0.5 EU/ml)

- Also in environment, including water

- Humans are orders of magnitude more sensitive to endotoxin than other mammals (mice models)

What is Endotoxin?

- Soluble endotoxin is released when
  - bacteria are destroyed AND during active cell growth
  - TIP: if endotoxin and TVC is high, kill your bugs first!

- Heat-stable, highly resistant to routine disinfection and sterilisation processes
  - glassware is rendered pyrogen-free by heating at 200-220^{o}C for 2-3 hours
  - plastics – gamma-irradiation
  - fluids - ultrafiltration

Illustration courtesy Charles River Endosafe, SC

How do we measure Endotoxin?

- In vivo test: Rabbit pyrogen
- In vitro test: *Limulus* amoebocyte lysate (LAL assays)
- Dependent on the blue-blooded Horseshoe crab donating haemolymph

vs Recombinant assays
- viable synthetic alternative
- crab-sparing, environmentally sustainable
- decreased batch to batch variation and no false positives from activation of alternative pathway by fungi/peptidoglycans

Endotoxin test result

- Pass or Fail (original gel clot LAL method)
  - Lab needs to know the limit is 0.25 or 30 EU/ml
- Quantitative (including Recombinant)
  - Point of care
  - NATA accredited laboratory
  - how much did I fail by?

Machine	Frequency	Required Tests	Test Frequency Reference	Required Test Reference
Supply Water **Batch-Washer and Manual Cleaning**	On commissioning	TVC ≤100cfu/100ml Endotoxin <=0.25 EU/ml	AS 4187 Amd2 Clause 7.2.3.1	AS 4187 Amd2 Table 7.2
	Monthly	TVC ≤100cfu/100ml Endotoxin <=0.25 EU/ml	AS 4187 Table 10.2	
Final Rinse Water **Batch-Washer and Manual cleaning**	Monthly for first 12 months then frequency may be adjusted to a minimum of annually provided test results remain with specification	TVC ≤100cfu/100ml	AS 4187 Amd2 Table 8.1	
	Annually – frequency may be adjusted (increased or decreased) according to test results to ensure they remain within the specifications (refer to A7.2.3.1 for guidance)	Endotoxin ≤0.25 EU/ml		

Machine	Frequency	Required Tests	Test Frequency Reference	Required Test Reference
Supply Water **AFER**	On commissioning	TVC ≤ 10cfu/100ml *Pseudomonas aeruginosa* Not Detected/100ml (Atypical) Mycobacterium Not Detected/100ml Endotoxin <= 30 EU/ml	AS 4187 Amd2 Clause 7.2.3.1	AS 4187 Amd2 Table 7.3
	Not Required - regular quarterly testing of supply water for AFERs has been removed from AS 4187 Table 10.3			AS 4187 Amd2 Table 10.3
Final Rinse Water **AFER**	Monthly	TVC ≤ 10cfu/100ml *Pseudomonas aeruginosa* ND/100ml (Atypical) Mycobacterium ND/100ml	AS 4187 Amd2 Table 8.1	AS 4187 Amd2 Table 7.3
	Annually	Endotoxin ≤ 30 EU/ml		

Acknowledgements

- Endoscopy staff
- Infection Control Officer and nursing staff
- Soluscope Technical Service
- PathWest Laboratory Medicine – Environmental Microbiology Unit, Pharmaceutical Testing Facility, Microbial Contamination Testing Laboratory
- Hospital engineer

Thank you

Additional sampling tips

- Medivators, Reliance EPS, others
  - Possibility of contamination of inside of container or lid
  - Certified endotoxin free container if performing endotoxin
  - Volume is greater under the new AS
    - 100ml for the TVC
    - 200ml for the mycobacteria (duplicate)
    - +/- 100ml Pseudomonas aeruginosa
    - ~10mls for Endotoxin

What you need from your laboratory

High Quality Service Delivery

- NATA Accredited 17025 (environmental laboratory)
- NATA Accredited culture and endotoxin methods (or working towards)
- Clear understanding of the test requirements and limits
  - Endotoxin detection range needs to cover 0.25 to 30 EU/ml
  - May wish to have a quantitative method
- Correct referenced methods (or other methods verified as equivalent)

What you need from your laboratory

High Quality Service Delivery

- Contactable during your full working day (interstate time differences)
- Early notification of out-of-specification results (needs to be negotiated as some labs may only send you a final report)
- Assistance with troubleshooting out-of-specification results, including specific media for specific organisms, epidemiological typing

What you need from your laboratory

Straight-forward practical procedures

- Step-by-step instructions on collection technique
- Easy access to sample bottles
- Sample bottles used for endotoxin testing must be endotoxin free (check with the manufacturer)
- Unambiguous request forms
- Reports get sent to the right people (including you?)
- Quick turn around time facilitated by
  - Onsite identification of organisms
  - Access to mycobacterial laboratory

Remediation concepts

- True or artefactual (collection technique, laboratory contamination)
- Systemic vs local water issue
- Supply vs machine/washer issue
- Supply – technical support, engineers
- Machine/washer issue – technical support, users, infection control
- Document whatever you do to have it as reference for next time
- Test going back through the system to know where the contamination is coming from

Remediation concepts

- Things you may discover along the way
  - Varying level of experience and competence with hospital water management (including your own)
  - Your technical support is invaluable
  - You might have low pressure, low flow, or both
  - You might need a pump
  - You might need more frequent changes of your filter banks eg monthly instead of 3 monthly
  - Cost is a consideration

Remediation concepts

- Must be a team based approach
- Get people into the same room
- Infection Control, CSSD/nurse manager of unit, engineer/facilities management, plumber, technical support