Public Health England

Protecting and improving the nation's health

External Quality Assessment (EQA) samples: should your laboratory be struggling to obtain the correct result?

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- Overview of why laboratories do EQA
- What can be learnt about challenging samples
- Findings from challenging samples/organisms
- Why participate?
- Summary of the benefits

Overview - Why laboratories do EQA

- To demonstrate competence as part of accreditation requirement – ISO/IEC 17025:2005 - *General requirements for the competence of testing and calibration laboratories*
- To help provide assurance of the results obtained provided they are treated and processed the same as other samples
- To help improve laboratory processes and understanding of regulation/legislation
- To remain up to date with new and emerging organisms - educational
- To challenge processes/media/training with difficult or atypical organisms
- To compare Inter-laboratory performance
- To support work tendered for as an accredited laboratory
- Because you enjoy the challenge that participating in EQA brings!

What can be learnt from challenging samples

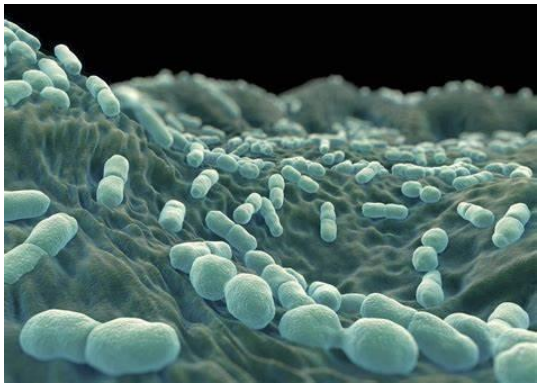
- Exposure to new organisms of public health concerns – raising awareness of their existence and allowing you to assess suitability of your current method/s or validating new ones
- Raising awareness of atypical organisms that exist in the environment and subsequent impact on laboratory testing and results
- Helps you to understand the limitations of methods/media used
- Helps you to understand the limitations of confirmation tests
- Allows you to understand gaps in your procedures – especially if an approved method is not followed

Findings from challenging organisms included in our EQA schemes

Standard scheme – S0701 (January 2021)

Sample contents and cfu levels per mL/g:

- *Bacillus cereus* ($3.7x10^4$) (wild strain)
- *Bacillus circulans* ($2.2x10^2$) (wild strain)
- *Staphylococcus aureus* ($4.0x10^4$) (wild strain)
- *Citrobacter braakii* ($4.3x10^3$) (wild strain)
- *Staphylococcus sciuri* ($2.9x10^4$) (wild strain)

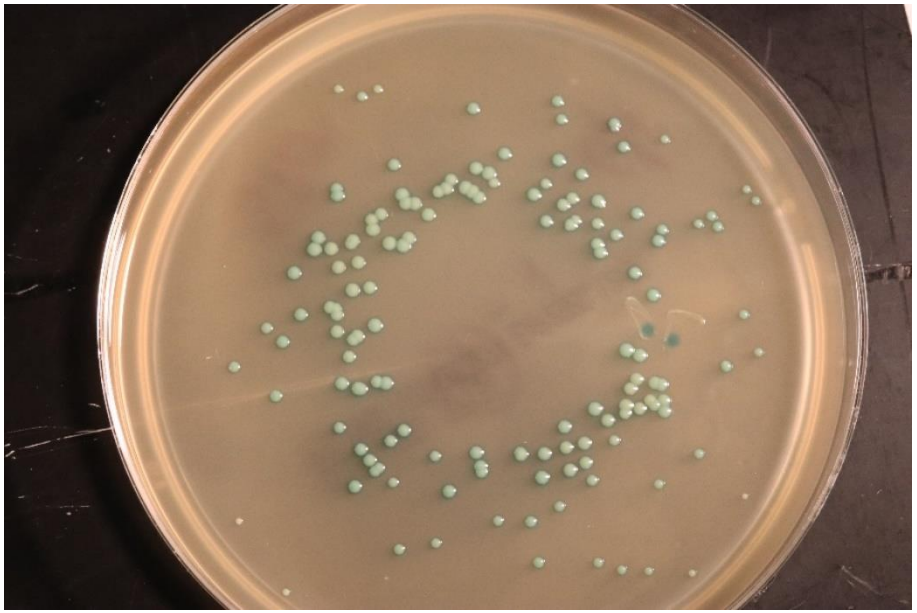


Examination: *Listeria* spp. including *monocytogenes*

29/103 (28%) of the participants reported a false positive result for this examination when the sample did not contain a *Listeria* spp.

121/123 (98%) reported that *Listeria monocytogenes* was absent from the sample

S0701– image



Bacillus cereus

Bacillus circulans

Staphylococcus aureus

Citrobacter braakii

Staphylococcus sciuri

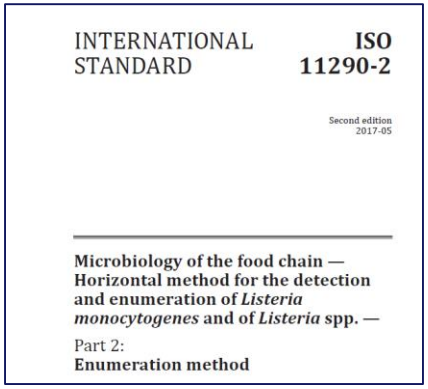
The sample contained a *Staphylococcus sciuri* and in the FEPTU laboratory this organism grew as 0.1 – 0.5mm circular entire blue/green colonies on Agar *Listeria* according to Ottaviani and Agosti (ALOH)

Confirmation tests

ISO 11290-2:2017 - Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. — Part 2: Enumeration method states

Section 9.3.3 ‘Consider as presumptive *Listeria* spp. the blue-green colonies with or without opaque halo’

Section 9.4.3 of the ISO method details the numerous confirmation tests that can be carried out for *Listeria* spp.


INTERNATIONAL STANDARD

ISO 11290-2

Second edition
2017-05

Microbiology of the food chain — Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. —

Part 2:
Enumeration method

Table C.1

Table C.1 — Distinction of *Listeria* spp. from other bacterial genera or species

	Gram appearance	Catalase	Motility (20 °C to 25 °C)	VP test	Growth at 37°C
Listeria spp.	Gpb/Gpcb	+	+	+	+
Bacillus spp. [a]	Large Gpb[b] spore bearer (young culture)	+	V	V	+
Carnobacterium spp. [a]	Gpb	-	-	-	+
Staphylococcus spp.[a]	Gpc	+	-	V	+
Streptococcus spp. [a]	Gpc	-	-	V	+

This strain of *S. sciuri* was Voges–Proskauer (VP) negative

Legionella Isolation scheme – G121B (April 2021)

Sample content and cfu levels per litre:

- *Legionella bozemanii* ($3.1x10^4$) (wild strain)
- *Legionella pneumophila* serogroup 1 ($2.2x10^5$) (wild strain)
- *Escherichia coli* ($2.5x10^3$) (wild strain)

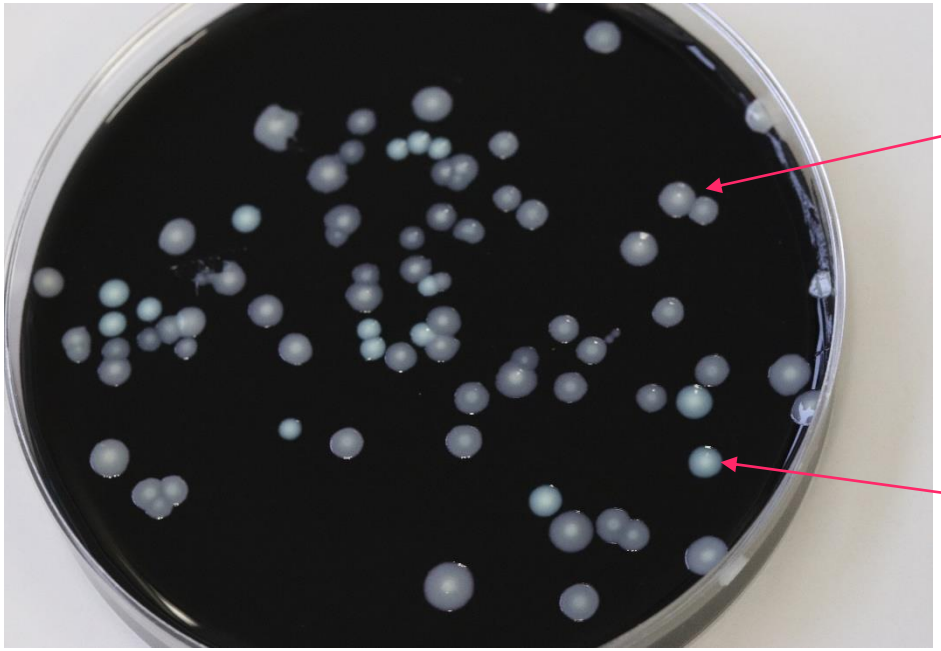
162/169 (96%) of the laboratories reported a ‘detected’ result for *Legionella* spp.

Eight of these laboratories did not provide an identification or serogroup of the *Legionella* spp. isolated

27/154 (18%) of the laboratories failed to report that two species of legionellae were in the sample

G121B - images

In the FEPTU laboratory, two types of colonies were observed on glycine, vancomycin, polymyxin B and cycloheximide (GVPC) media after processing. Incubation was aerobic, 37^{o}C read at 3, 7 and 10 days

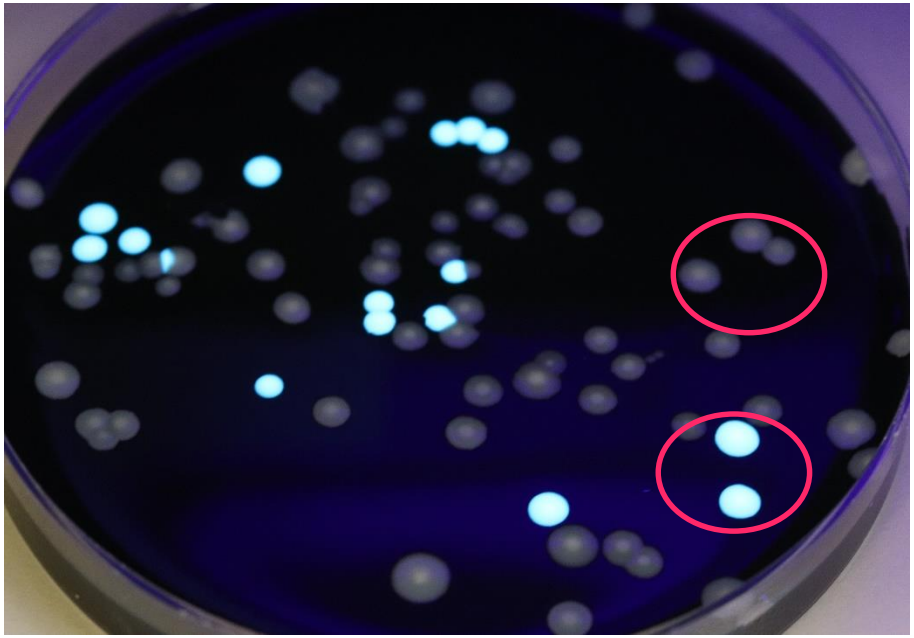


One colony type was **2-3mm circular, grey flat with ground-glass appearance** and did not fluoresce under a UV light (*L. pneumophila*)

The second colony type was **2-3mm circular, shiny bluish flat with ground-glass appearance** and produced a white fluorescent under a UV light (*L. bozemanii*)

G121B – images under a UV light

In the FEPTU laboratory, two types of colonies were observed on glycine, vancomycin, polymyxin B and cycloheximide (GVPC) media after processing. Incubation was aerobic, 37^{o}C read at 3, 7 and 10 days



One colony type was 2-3mm circular, grey flat with ground-glass appearance and **did not fluoresce** under a UV light (*L. pneumophila*)

The second colony type was 2-3mm circular, shiny bluish flat with ground-glass appearance and produced a **white fluorescent** under a UV light (*L. bozemanii*)

Breakdown of results

Breakdown of results reported by 127 of the laboratories for the second species

Reported result	Count	%
Legionella spp. not *L. pneumophila*	78	61
Legionella spp.	25	20
L. bozemanii	20	16
L. anisa	3	2
L. micdadei	1	-

Legionella information

Legionella infection occurs mainly by inhalation of aerosols generated from water sources such as distribution systems and cooling towers

The species *Legionella pneumophila* accounts for about 90% of the cases of legionellosis, and about 85% are due to serogroup 1

Other *Legionella* species are rarely pathogenic in humans, the most common being *L. longbeachae* (3.9%) and *L. bozemanii* (2.4%)

ISO 11731:2017 Table A.1 does list *L. bozemanii* associated with disease

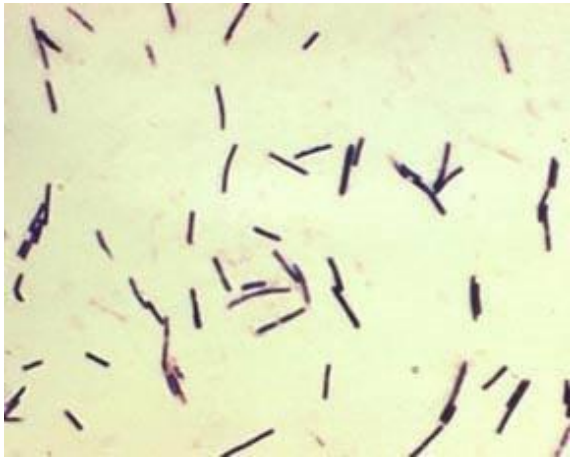
Table A.1 — *Legionella* species associated with disease

L. anisa	*L. erythra*	*L. longbeachae*	*L. pneumophila*
L. birminghamensis	*L. feeleii*	*L. lytica*	*L. sainthelensi*
L. bozemanii	*L. gormanii*	*L. maceachernii*	*L. steelei*
L. cardiaca	*L. hackeliae*	*L. micdadei*	*L. tusconensis*
L. cincinnatiensis	*L. jordanis*	*L. nagasakiensis*	*L. wadsworthii*
L. clemensonensis	*L. lansingensis*	*L. oakridgensis*	
L. dumoffii	*L. londiniensis*	*L. parisiensis*	
NOTE In addition, *L. waltersii* has been detected by polymerase chain reaction (PCR) from a clinical sample.			

Drinking Water Scheme – W193B (August 2020)

Sample contents and approximate cfu per 100mL:

- *Klebsiella pneumoniae* (33) (wild strain)
- *Escherichia coli* (43) (wild strain)
- *Pseudomonas aeruginosa* (31) (wild strain)
- *Clostridium sordellii* (35) (wild strain)
- *Staphylococcus aureus* (54) (NCTC 8178)



Clostridium perfringens examination:

19/99 (19%) of the participants reported a false positive result for this examination. This sample contained a *Clostridium sordellii* at approximately 35 colony forming units per 100mL

In the FEPTU laboratory, this organism grew on tryptose-sulfite-cycloserine (TSC) agar as <0.5mm circular greyish/black colonies following anaerobic incubation at 44^{o}C for 24 hours

Methods used

It is clear from the returned results that laboratories are not undertaking confirmation tests as required. Laboratories following ISO 14189:2013 Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration should carry out an **acid phosphatase test**

Organism	Buffered nitrate motility medium		Lactose-gelatin medium		Acid phosphatase
	Motility	Nitrate reduction	Lactose fermentation	Gelatin liquification	
C. perfringens	Non-motile	Positive	Positive	Positive	Positive
C. sordellii	Motile	Negative	Positive	Positive	Negative

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Methods used - 1

It is clear from the returned results that laboratories are not undertaking confirmation tests as required. Laboratories following ISO 14189:2013 Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration should carry out an **acid phosphatase test**

Organism	**Buffered nitrate motility medium**		**Lactose-gelatin medium**		**Acid phosphatase**
	Motility	Nitrate reduction	Lactose fermentation	Gelatin liquification	
C. perfringens	Non-motile	Positive	Positive	Positive	Positive
C. sordellii	Motile	Negative	Positive	Positive	Negative

Methods used - 2

All the confirmation tests shown in the table are listed in the Standing Committee of Analysts method manual - The Microbiology of Drinking Water (2015) – Part 6 – Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration

Organism	Buffered nitrate motility medium		Lactose-gelatin medium		Acid phosphatase
	Motility	Nitrate reduction	Lactose fermentation	Gelatin liquification	
C. perfringens	Non-motile	Positive	Positive	Positive	Positive
C. sordellii	Motile	Negative	Positive	Positive	Negative

http://standingcommitteeofanalysts.co.uk/library/MoDW%20Part%206%20-%20Clostridia%20(PUBLICATION%20July%202015).pdf

Methods used – 2

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	Motility	Nitrate reduction	Lactose fermentation	Gelatin liquification	
C. perfringens	Non-motile	Positive	Positive	Positive	Positive
C. sordellii	Motile	Negative	Positive	Positive	Negative

http://standingcommitteeofanalysts.co.uk/library/MoDW%20Part%206%20-%20Clostridia%20(PUBLICATION%20July%202015).pdf

Should your laboratory be struggling to obtain the correct result?

Sample S0701: *Listeria* spp.: No, if you follow the ISO method and do the right confirmation tests

Sample G121B: *Legionella bozemanii*: No, if you follow the ISO method, closely check for different types of colonies and then check them under a UV light

Sample W193B: *Clostridium perfringens*: No, if you follow published methods and do the correct confirmation tests

Why participate?

PHE PT samples are designed to challenge your testing procedures therefore will include challenging organisms – so beware

We extensively test the samples using ISO methods – so your results should align with our results including confirmatory test results

Process PT samples the same as other routine samples. Otherwise nothing will be learnt about your quality system

We are not here to trick you but to:

- raise awareness of the limitation/s of your procedure or method
- encourage the use of approved methods
- endorse the requirement to carry out confirmatory tests
- give you an opportunity to examine samples containing organisms less frequently encountered that are of public health concern
- provide an insight into staffs' knowledge and experience

Summary of the benefits to you

Provides evidence of your laboratory's competency for accreditation

Raises awareness of new and emerging organisms of public health concern

Provides an opportunity to improve staffs' knowledge and experience with organisms not frequently encountered

Better understanding of your performance in relation to the method/s you use

Greater understanding of the limitation/s of your method especially with atypical/emerging organisms

Highlights impact on results when approved methods are not followed

Highlights impact on results with unusual, atypical strains or less commonly encountered organisms

Gives you an independent assessment of your laboratory's overall performance

Allows you to identify gaps in your process where quality improvements are required

Acknowledgements

- Angela Appea
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Thank you for listening