UK Health Security Agency

Common challenges for FEPTU participants – is it a fantasy?

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- Some facts and figures
- Examples of challenging organisms
- Advice
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Overview – why laboratories do PT?

- To demonstrate competence as part of accreditation requirement – ISO/IEC 17025:2005 - *General requirements for the competence of testing and calibration laboratories*
- Helps to provide assurance of the results obtained provided they are treated and processed the same as other samples
- Helps 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
- Inter-laboratory comparison of performance
- To support work tendered for as an accredited laboratory
- Because you enjoy the challenge and the educational value that participating in PT brings!

What can you learn from challenging samples

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

Facts and figures

- Number of Schemes
  18
- Number of participants
  752
- Number of countries
  62

UK Health Security Agency

Commonly observed challenges

Clostridium perfringens

- This specific examination is part the following schemes:
  - Food: Standard
  - Water: River, lake or stream (recreational) and drinking
  - In bottled and mineral (BMW) - sulfite reducing anaerobic bacteria

	Food	Water
Media used	Sulfite-cycloserine agar (SC)	Tryptose sulfite cycloserine agar (TSC agar)
Confirmation tests	*ISO 7937:2004 Lactose, gelatin and nitrate motility	**ISO 14189:2013 Acid phosphatase ±Standing Committee of Analysts: Acid phosphatase, buffered nitrate motility medium

- *ISO 7937:2004 - Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of *Clostridium perfringens* —Colony-count technique
- **ISO 14189:2013 Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration
- ±Standing Committee of Analysts (SCA): The Microbiology of Drinking Water (2015) – Part 6 – Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration

Clostridia (performance %)

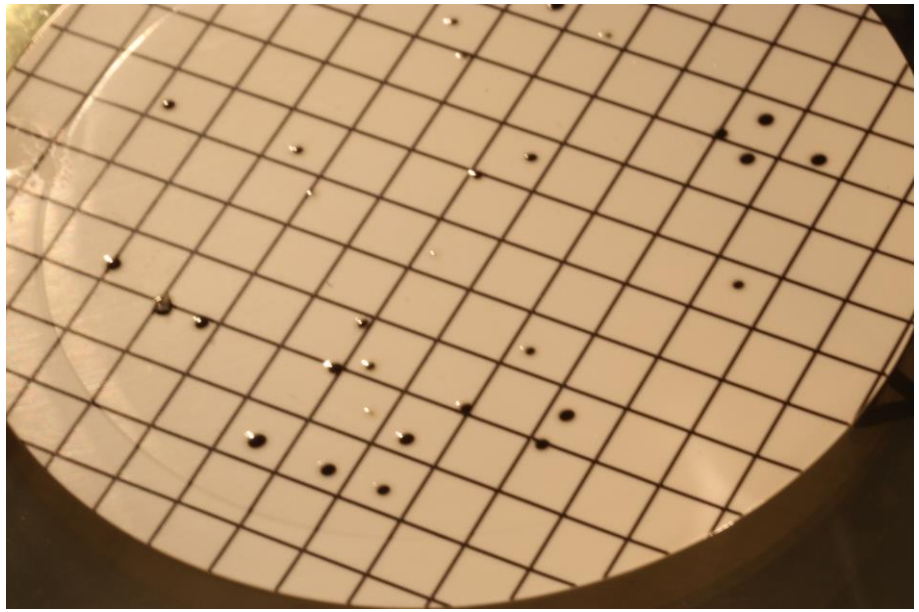
- Over the last four samples containing this organism

Food		Water	
C. perfringens	Other species	*C. perfringens*	Other species
Standard: 99	-	Drinking: 97	78
		Recreational: 96	-
		BMW: 97	68

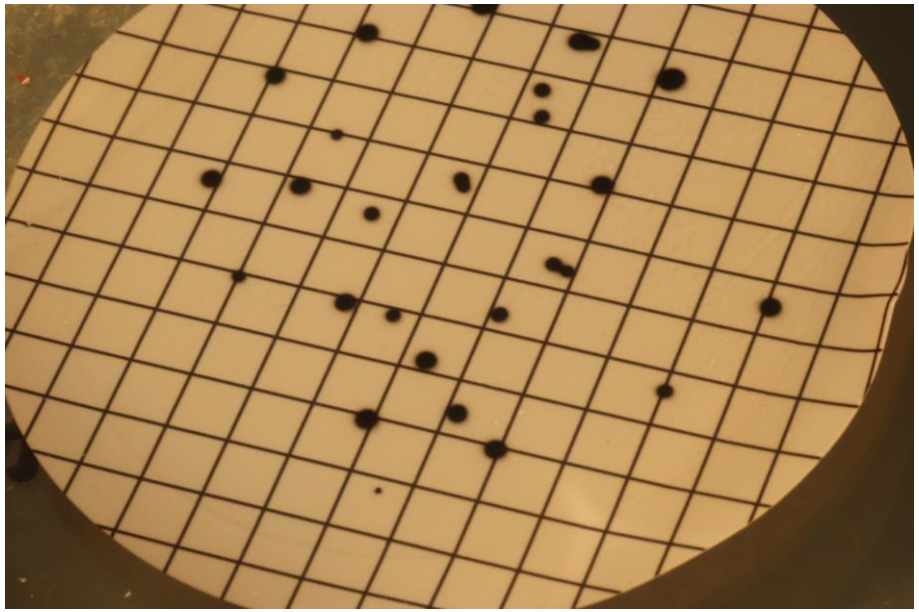
W204A - examination *C. perfringens*

- 21/62 (34%) of the participants reported a false positive result for this examination
- This sample contained a *Clostridium bifermentans* at approximately 16 colony forming units per 100mL
- In the FEPTU laboratory this organism grew on TSC agar as 1-2 mm circular black opaque colonies following anaerobic incubation at 44^{o}C for 24 hours

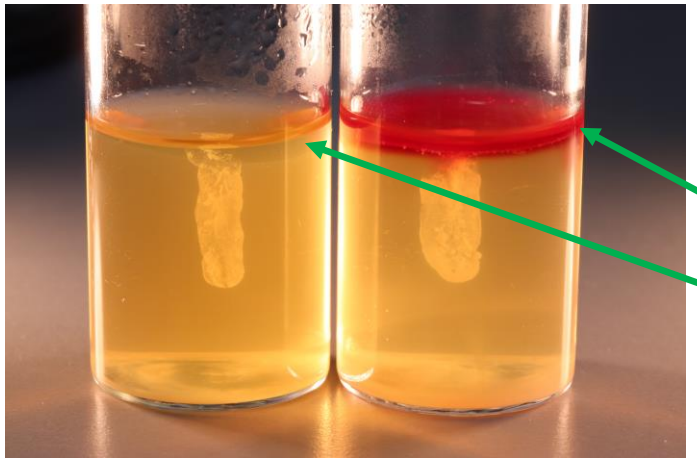
No overlay



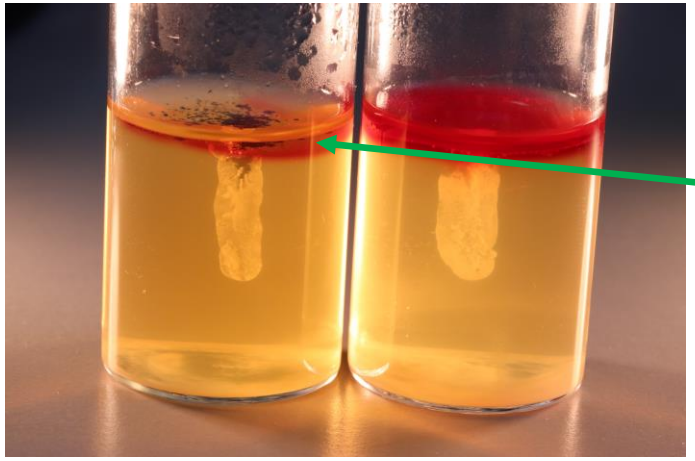
With an overlay



W204A - examination *C. perfringens*



Nitrate:
C. perfringens positive (control)
C. bifermentans negative

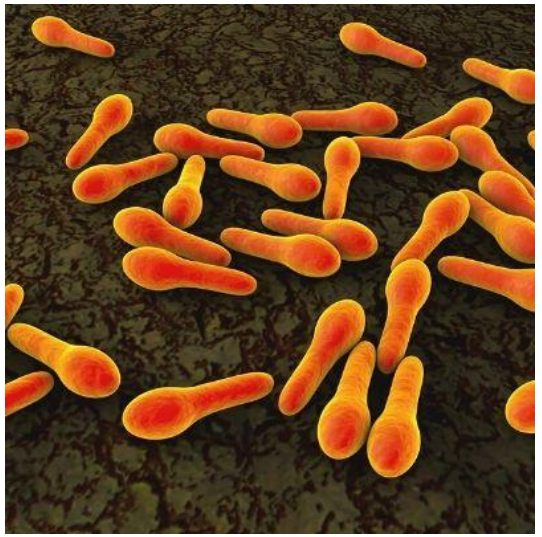


Adding zinc – *C. bifermentans* nitrate is still negative

This confirmation test would have confirmed the organism is not a *C. perfringens*

Clostridia confirmation tests summary

Organism	Motility	Nitrate reduction	Lactose fermentation	Gelatin liquification	Acid phosphatase
C. perfringens	Non-motile	Positive	Positive	Positive	Positive
C. bifermentans	Motile	Negative	Positive	Positive	Negative
C. sordellii	Motile	Negative	Positive	Negative	Negative
C. subterminale	Motile	Negative	Positive	Positive	Negative



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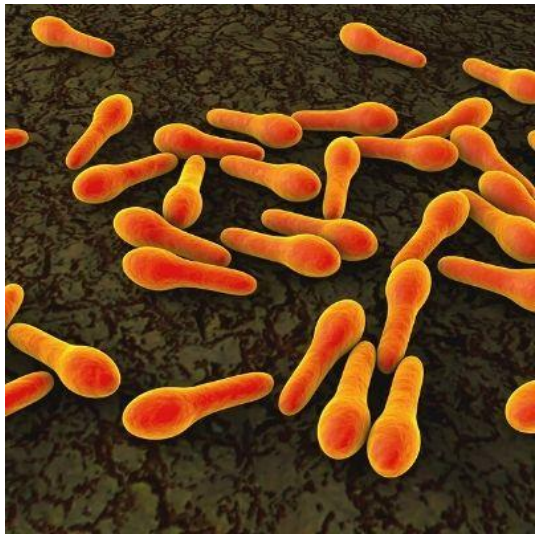


Pseudomonas aeruginosa

- This specific examination is part the following schemes:
  - Food: non-pathogen as *Pseudomonas* species
  - Water: swimming pool/hydrotherapy, drinking, bottled and mineral and hospital tap

	Food	Water
Media used	PCFC/CFC (Pseudomonas CFC agar containing cetrimide, fucidin and cephaloridine)	PCN (Pseudomonas Cetrimide agar)
Confirmation tests	Oxidase	*ISO 16266:2006 Florescence, oxidase test, acetamide broth, and King's B media **Standing Committee of Analysts Florescence, oxidase, milk agar with cetyl trimethylammonium bromide (MCA)

- *ISO16266:2006 - Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane filtration
- **Standing Committee of Analysts (SCA): The Microbiology of Drinking Water (2015) – Part 8 – Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa*

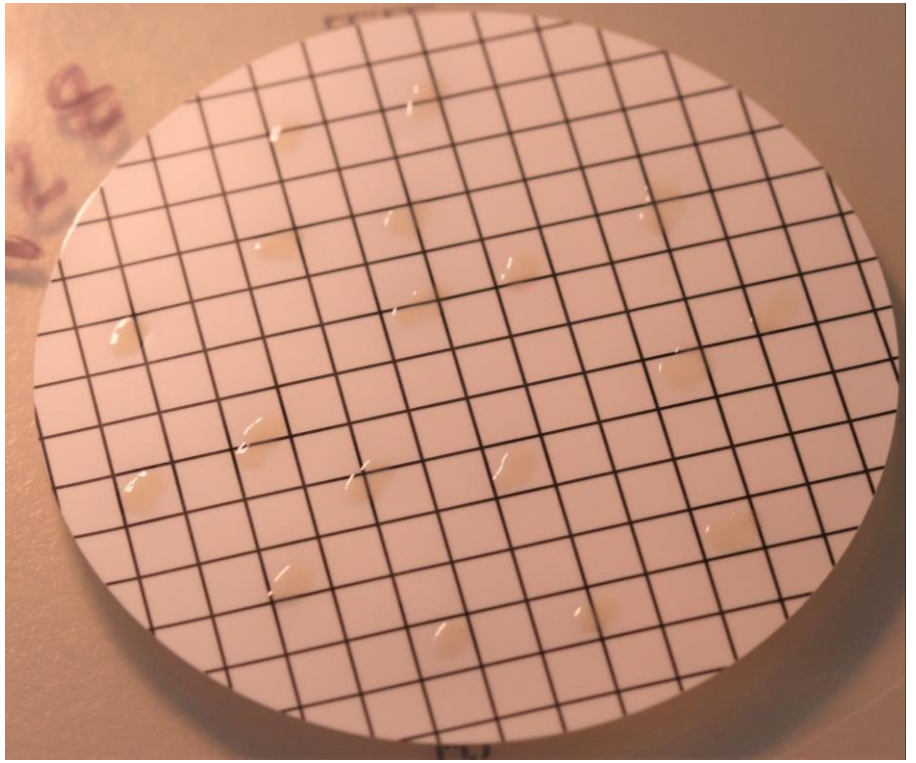
P. aeruginosa (performance %)

- Over the last four samples containing this organism

Food - species		**Water – *P. aeruginosa***	
P. aeruginosa	Other species	*P. aeruginosa*	Other species
98	94	Drinking: 99	-
		HTW: 100	-
		BMW: 99	-
		Swimming: 99	-

Old sample performance – *P. aeruginosa*

- 15/110 (14%) of the laboratories reported a false negative result
- The sample contained a *P. aeruginosa*; the participants' median was 34 colony forming units (cfu) per 100mL
- In the FEPTU laboratory this strain produced pale, flat opaque colonies on PCN, Confirmatory tests results on MCA were positive for growth and casein hydrolysis, fluorescence and for pyocyanin production
- ISO 16266 section: 8.4 states to 'count all non-pyocyanin producing colonies that fluoresce as presumptive *Pseudomonas aeruginosa* and confirm their identity using acetamide broth'
- However, on confirmation test with acetamide broth, this strain does not produce ammonia
- Therefore, the reporting of a negative result for this examination was considered correct and scores were amended to reflect this



- For information, only 38% of non-pyocyanin-producing strains of *P. aeruginosa* will produce a positive result in acetamide. Further biochemical testing may be necessary for definitive identification

What does published methods state

- ISO 16266:2006
  - Count all colonies that produce blue/green (pyocyanin) colour as confirmed *Pseudomonas aeruginosa*
  - Count all non-pyocyanin producing colonies that fluoresce as presumptive *Pseudomonas aeruginosa*
  - Count all other reddish brown pigmented colonies that do not fluoresce as presumptive *Pseudomonas aeruginosa*
  - ➢ confirm their identity using the oxidase test, acetamide broth, and King's B media

- SCA – part 8
  - Colonies may be blue-green, greenish brown or brown in colour. Also, examine the filter under the UV lamp and count all fluorescent colonies. These colonies, which may or may not be pigmented, should also be considered as presumptive *Pseudomonas aeruginosa*
  - Colonies which are 2 - 4 mm in diameter and show typical pigment production and possess an "area of clearing" around the colony where the casein has been hydrolysed are recorded as confirmed *Pseudomonas aeruginosa*

Enterococci

- This specific examination is part the following schemes:
  - Food: Non-pathogen
  - Water: All recreational, drinking, and bottled and mineral

	Food	Water
Media used	Slanetz and Bartley (S+B) agar	Slanetz and Bartley (S+B) agar
Confirmation tests	BS 4285-3:1985 (old method) Bile Aesculin agar (BAA)	*ISO 7899-2:2000 Bile-aesculin-azide agar ±Standing Committee of Analysts: Bile aesculin agar (BAA) or Kanamycin aesculin azide agar (KAAA) (catalase)

- *ISO 7899-2:2000 - Water quality — Detection and enumeration of intestinal enterococci — Membrane filtration method – part 2: Membrane filtration method
- ±Standing Committee of Analysts (SCA): The Microbiology of Recreational and Environmental Waters (2015) – Part 4 – Methods for the isolation and enumeration of enterococci

Enterococci (performance %)

- Over the last four samples containing this organism

Food	Water
89	Drinking: 99.5
	Recreational: 99.5
	Bottled and mineral: 97

S102A

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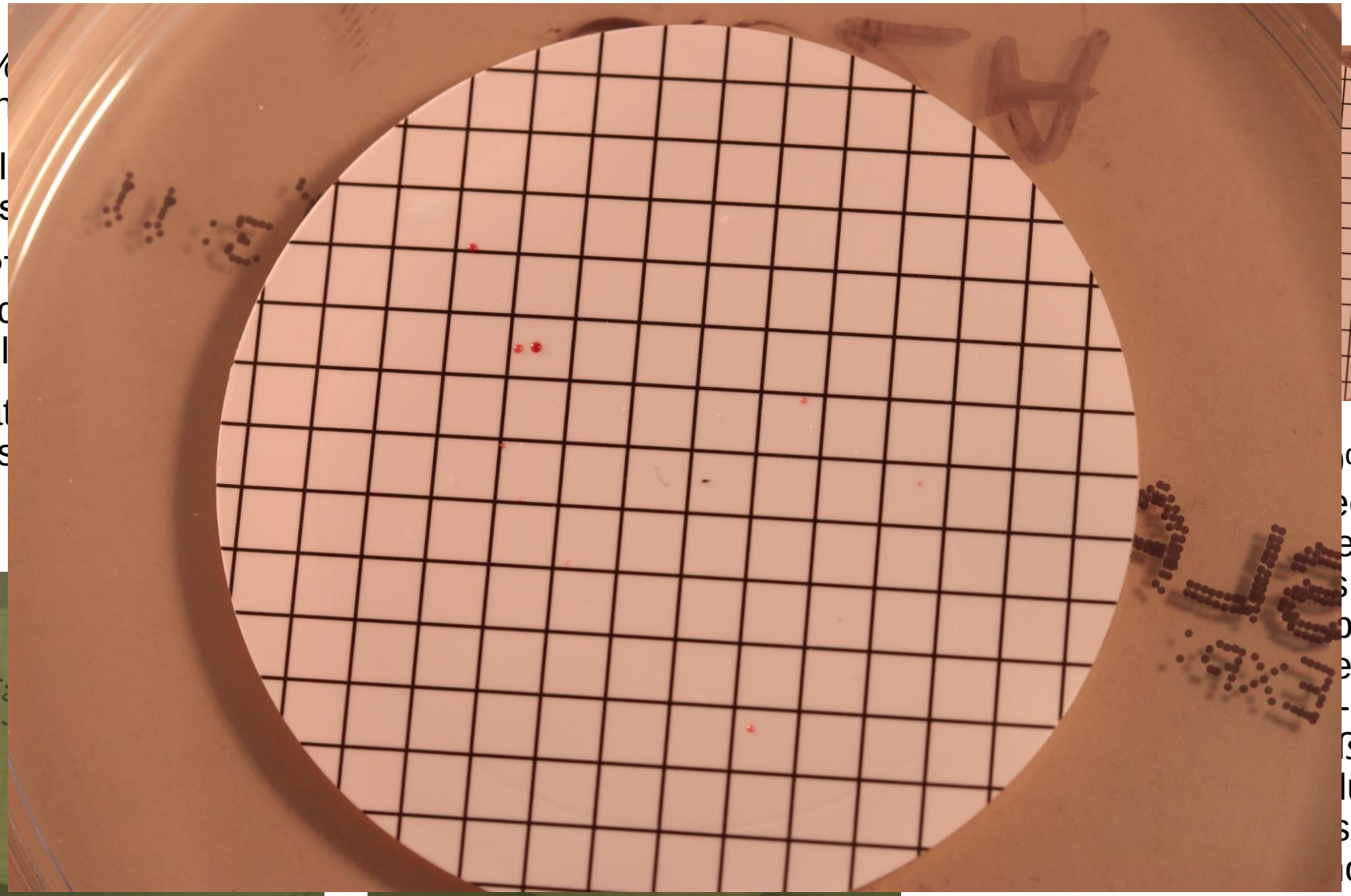

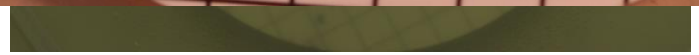

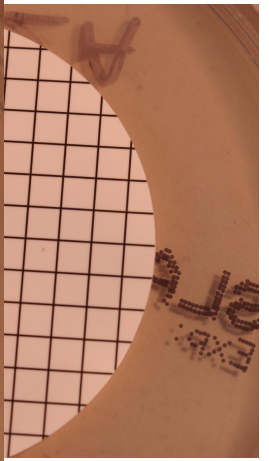

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False positives

- S107B – Enterococci
  - 8/55 (15%) of the participants reported a false positive result for this examination
  - The sample did not contain an *Enterococcus*
  - The *Escherichia coli* in this sample can grow on Slanetz-and Bartley (S+B) agar as small red/maroon colonies
  - A confirmation test such as bile esculin would have confirmed that these colonies were not an enterococci

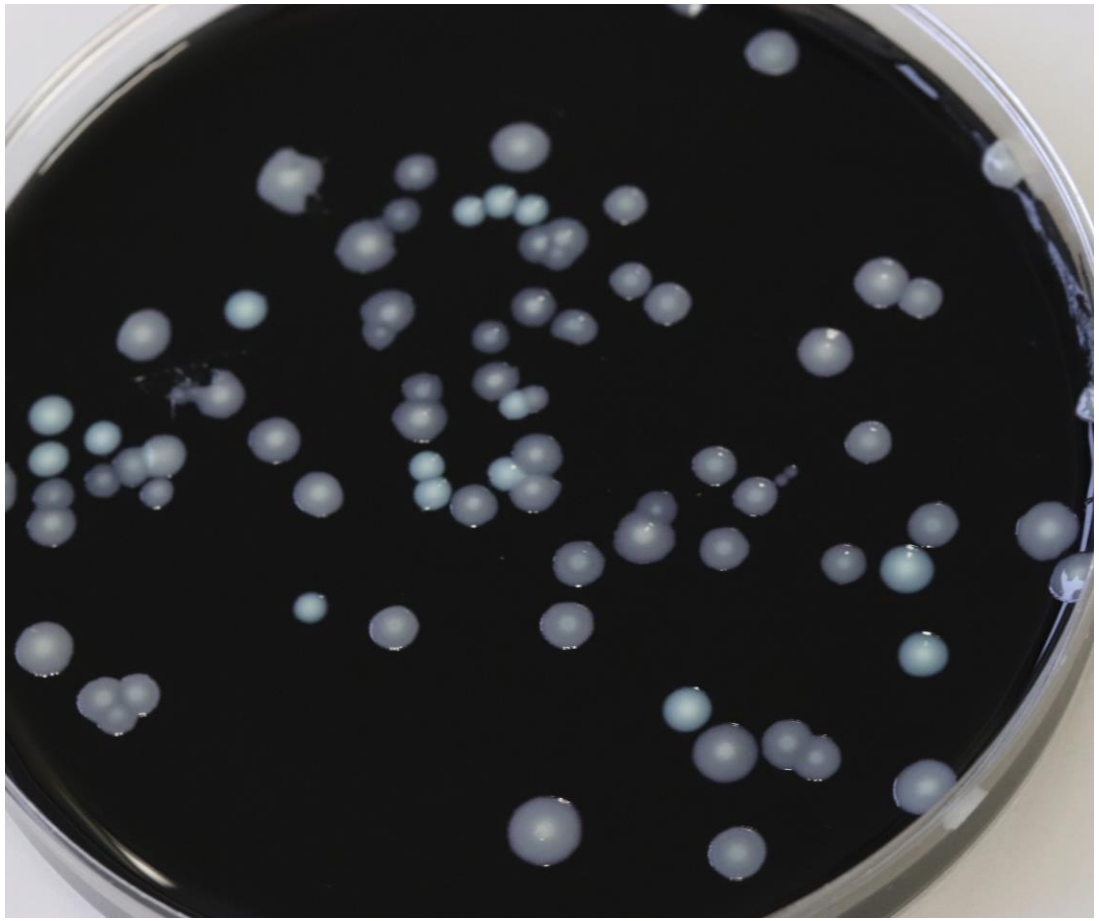
False positives

- S105B – Enterococci
  - 9/82 (11%) of the laboratories reported a false positive result for this examination
  - The sample did not contain an *Enterococcus*
  - The sample contained an *Aerococcus viridans* that may have grown on Slanetz and Bartley (S+B) agar as pinpoint reddish colonies
  - These colonies also gave a positive result with the bile esculin confirmation test
  - Additional tests can be carried out if there is any doubt about the organism grown.
  - For this examination laboratories reporting a false positive result have been awarded a score of 2

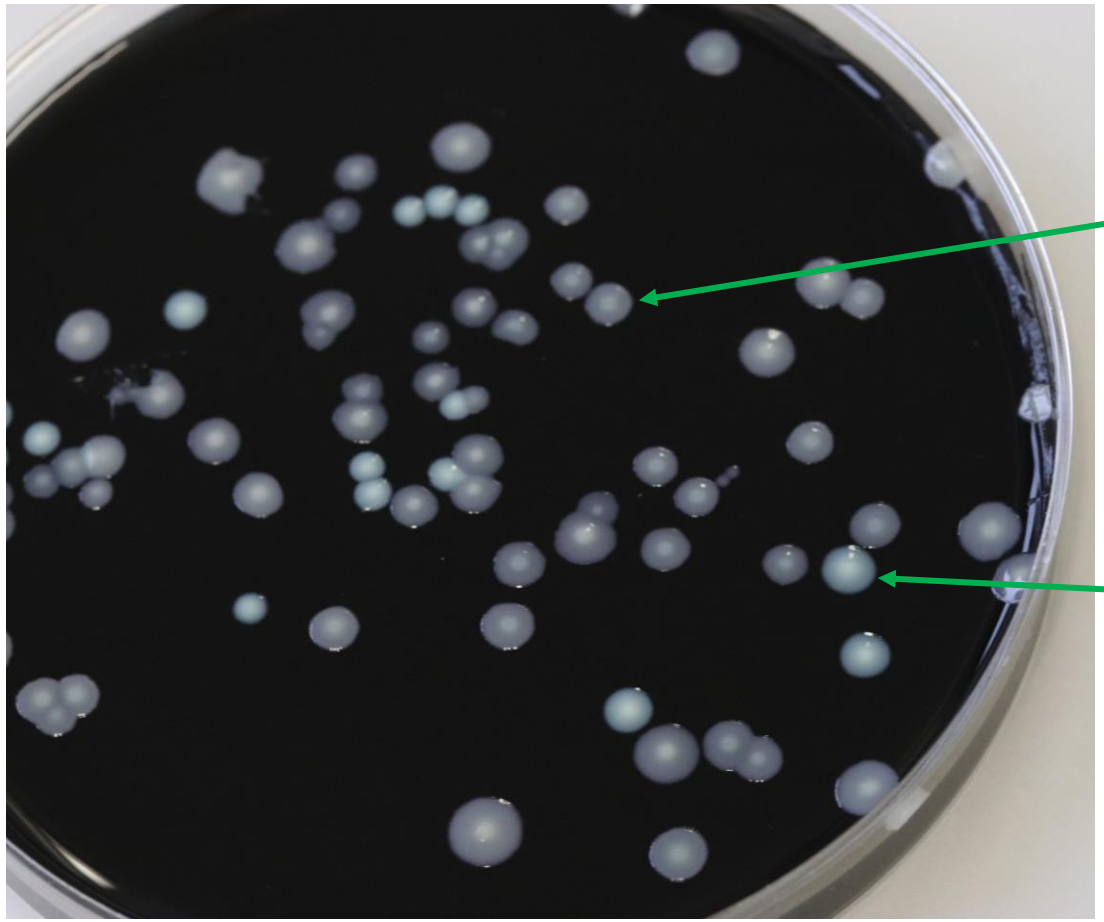
Legionellae – G121B (February 2021)

Content: *Legionella bozemanii (*3.1x10^4), *Legionella pneumophila* serogroup 1 (2.2x10^5) and *Escherichia coli* (2.5x10^3)

- 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
- 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

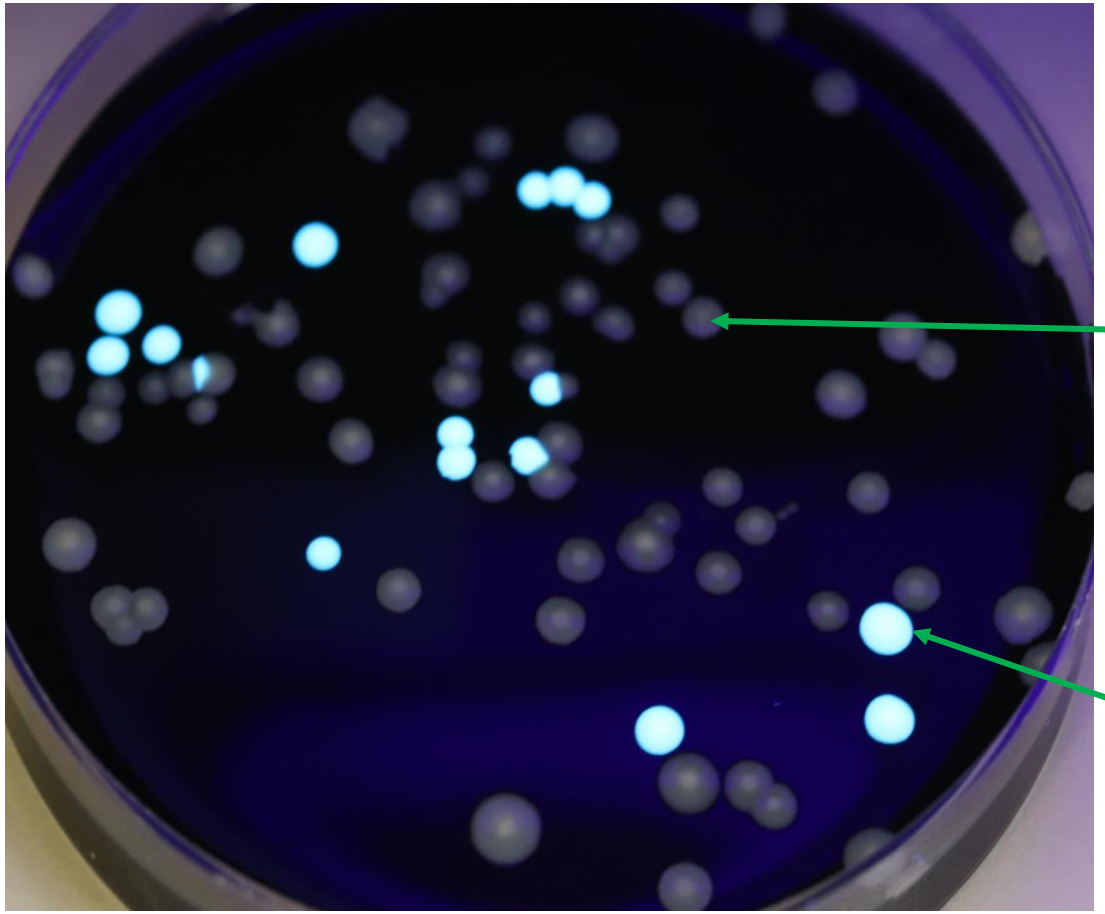


G121B - GVPC



- 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 - GVPC



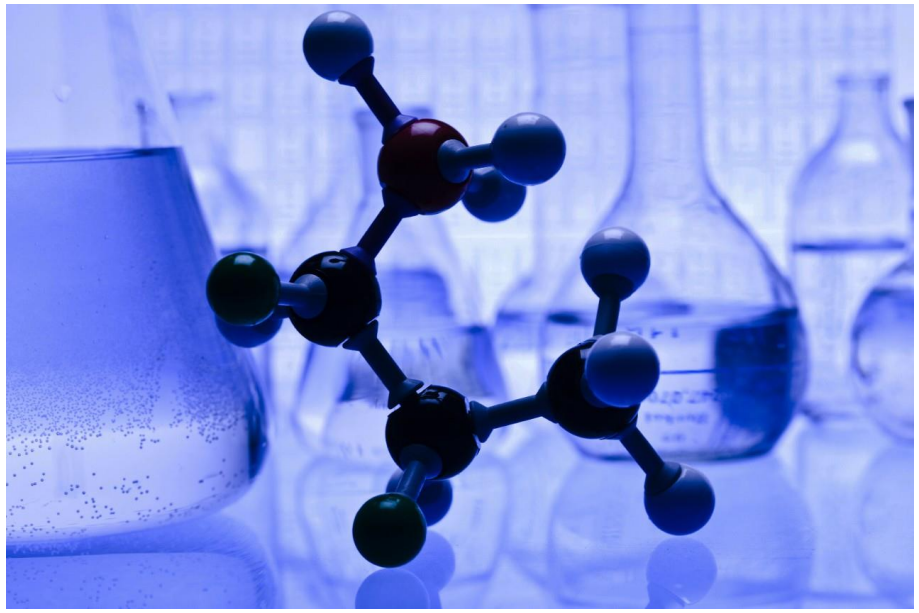
- One colony type was 2-3mm circular, grey flat with ground-glass appearance and did not fluoresce under a UV light (*L. pneumophila*)
- The second colony type was 2-3mm circular, shiny bluish flat with ground-glass appearance and produced a white fluorescent under a UV light (*L. bozemanii*)

Advice

- If confirmation tests are not done, then report your result as presumptive so scores are awarded accordingly, this information can be provided in the comment section when returning results
- Use a magnifying glass to examine plates
- Adhere to your own procedure for media, incubation temperature and timings
- Only do confirmation tests you would do on real samples
- You can use alternative identification methods as long as they have been validated for use
- When you have a failure with a PT, investigate, request a repeat sample from us

General observations with PT

- There is a definite trend to use different methodologies for examining samples i.e. MPN method especially for coliform and *Escherichia coli*
- There is a trend to use more automated systems for identification work
- Wide variable results are observed with quantification results, more work needs to be done as root cause could be multiple reasons, such as training, assays, platforms etc.



PT updates

- Building a new website for FEPTU with a new URL – be more informative and easier to use
- Building a new PT software using the latest technology and ensuring compliance with Government Digital Service, participants will have more control on updating their laboratory information and access to the PT database
- Exploring expanding the current scheme portfolio to support:
  - Legislation changes such as coliphage testing
  - The drive in other water testing being done such a sewage
- One change you will see from April 2023 is the addition of colony count at 37^{o}C / 48 hours for swimming pool waters
- We are going to be more visible in the food and water arena
- Exploring the expansion of three analyst testing for more schemes

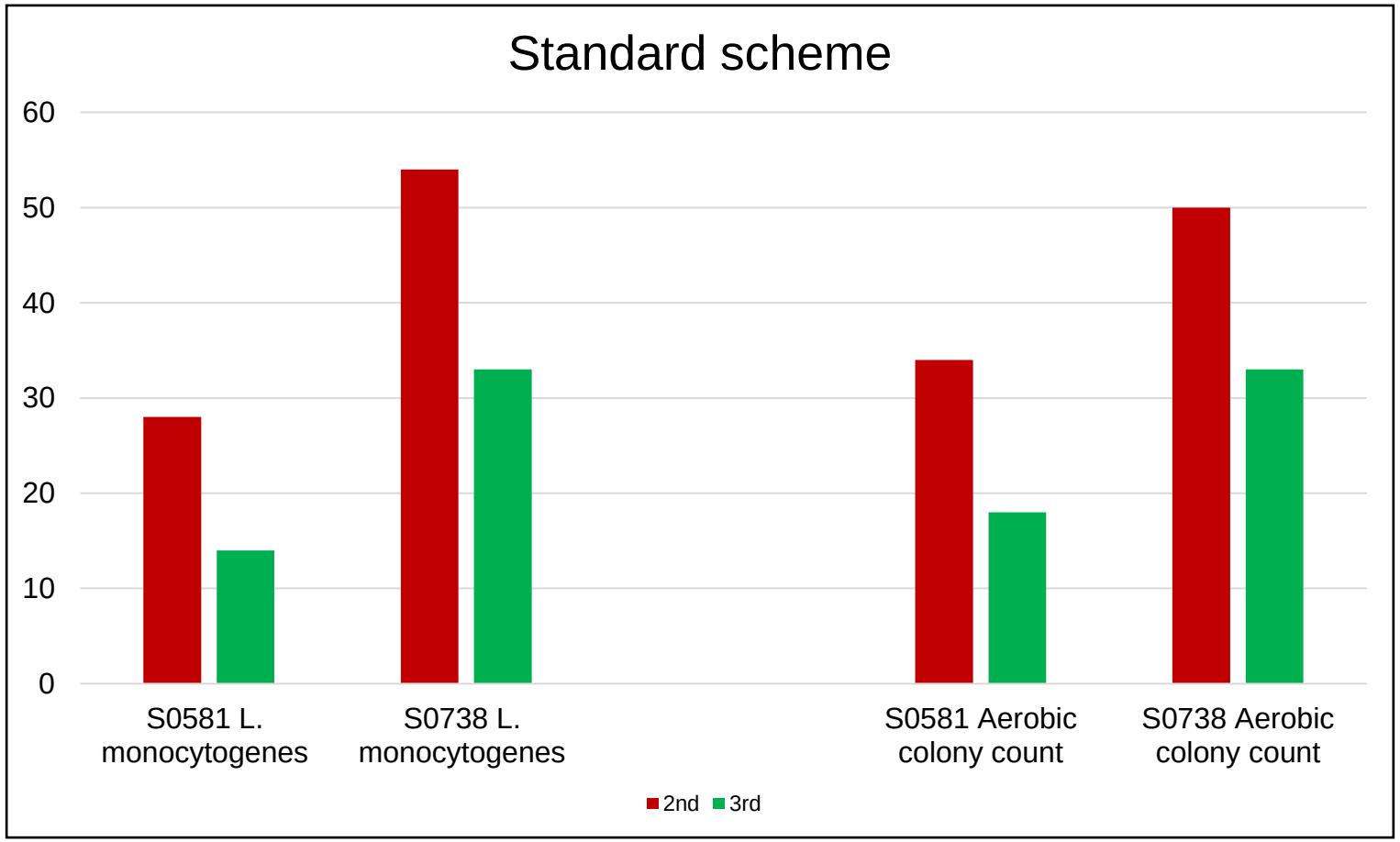
Three analyst reporting

- Introduced in the Standard Scheme in 2015
- Six schemes have the option for laboratories to report up to three results
- However, you have to nominate one result for use in the statistical analysis
- Z-score is provided for result 2 and 3
- You decide if you want to report more than one result, you can decide which examinations you want to do this for

- Is this option being used? Did an analysis on selected examinations from the first distribution and the latest distribution to compare the data

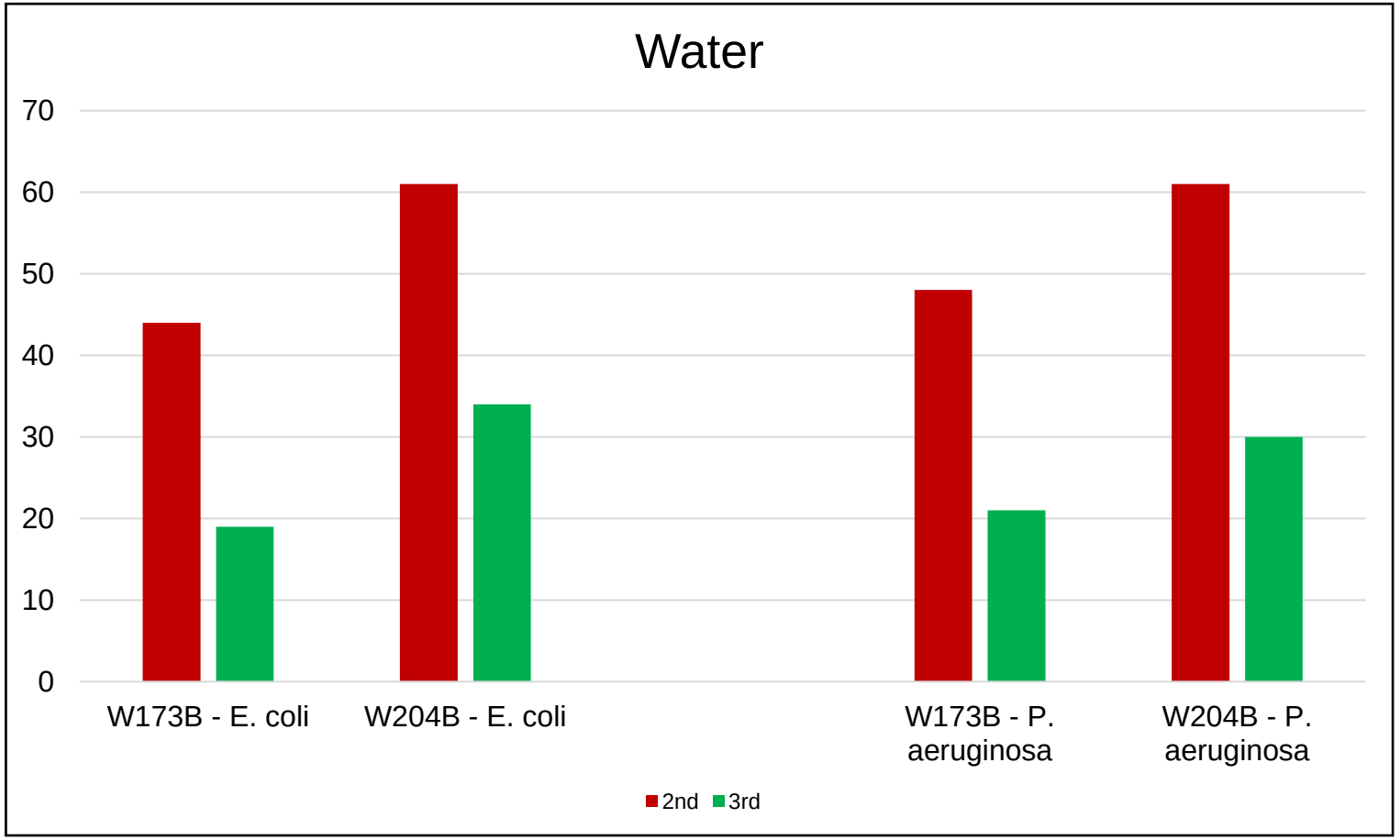
Three result reporting

- Food – S0581 (September 2015) and S0738 (July 2022)



Three result reporting

- Water – W173B (January 2017) and W204B (September 2022)



Conclusions

- UKHSA PT samples are designed to challenge your testing procedures therefore we will include challenging organisms – so beware
- We extensively test the samples using published/ISO methods – so your results should align with our results if the same including any confirmatory tests
- 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
  - provide an insight into your staffs' knowledge and experience
  - endorse the requirement to carry out confirmatory tests
  - encourage the use of approved methods
  - give you an opportunity to examine samples containing organisms less frequently encountered that are of public health concern

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- UKHSA

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Thank you for listening

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