UK Health Security Agency

What is challenging our PT participants?

Nita Patel

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Overview – why laboratories do PT?

- To demonstrate competence as part of accreditation requirement – ISO/IEC 17025:2017 - *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
  802
- Number of countries
  64

UK Health Security Agency

What are the challenges?

Standard Scheme S0786 - coliforms

Definition: bacteria which, at the specified temperature (i.e. 30°C or 37°C, as agreed) form characteristic colonies in crystal violet neutral bile lactose agar, and which in the confirmation test cause fermentation of lactose with the production of gas (in this case brilliant green lactose bile broth (BGBB))

Content: *Bacillus cereus* ($1.4x10^4$), *Staphylococcus aureus* ($1.1x10^4$), *Listeria monocytogenes* ($1.6x10^5$), *Leuconostoc mesenteroides* ($2.9x10^5$), *Enterobacter aerogenes* ($3.3x10^4$), *Citrobacter freundii* ($3.0x10^3$) – all levels per mL

For FEPTU our result ($3.1x10^3$ cfu g^{-1}) was 0.66 $\log_{10}$ cfu g^{-1} difference when compared to the participants' median ($1.4x10^4$ cfu g^{-1}) and fell outside the expected range ($4.3x10^3$ – $4.3x10^4$ cfu g^{-1}) – we initiated our own investigation to establish why? (criteria is <0.3 $\log_{10}$ difference and within range)

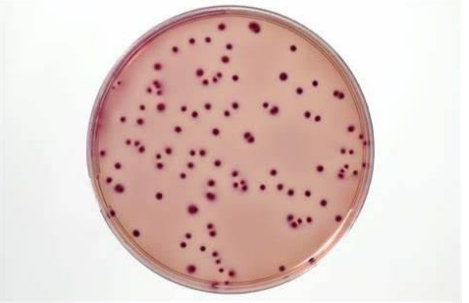
In the FEPTU laboratory two colony types were observed on violet red bile lactose (VRBL) following incubation at 37°C for 24 hours:

1. 1.2 mm purple round colonies (confirmed as *Enterobacter aerogenes*)
2. <0.5mm purple round colonies (confirmed as *Citrobacter freundii*)

ISO 4832:2006

- ISO 4832:2006 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique states *'purplish red colonies with a diameter of at least 0.5mm (sometimes surrounded by a reddish zone of precipitated bile) are considered as typical coliforms and do not require confirmation'*

- It also states '*to count and confirm atypical colonies (e.g. of smaller size)'*



Standard scheme S0786 - coliforms

Based on the statement within ISO 4832:2006 – in the FEPTU laboratory

For *E. aerogenes* no further confirmation test would be required

For the *C. freundii* colonies were <0.5mm therefore a BGBB was set up to test for gas formation. No gas was formed in the Durham tube; these colonies were excluded from the total coliform count. This may explain why FEPTU's result was outside the expected range and a 0.66 $\log_{10}$ difference observed when compared to the participants' median

I also checked our initial characterisation tests done on the *E. aerogenes* as full identification and confirmations are automatically done: the *E. aerogenes* did not produce gas in a Durham tube when BGBB was inoculated – therefore based on the definition in the ISO this would not be considered a 'coliform'

Three laboratories reported a low censored value – suspect that all colony types are confirmed

Why did laboratories not get the same results as FEPTU? – we sent out a questionnaire to better understand this issue

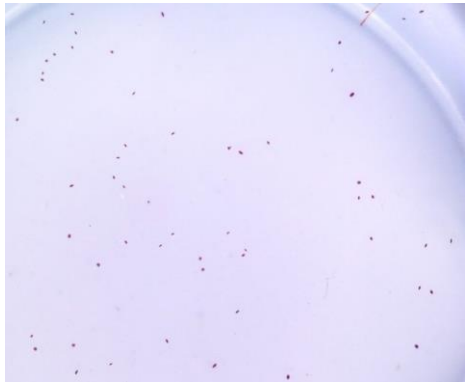
Standard scheme S0786 - coliforms

This questionnaire was sent to 63 laboratories. 29/63 (46%) provided a response

Of the 27 responses most laboratories 21/27 (78%) used ISO 4832: 2006 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique

Those who stated they follow ISO 4832:2006, 19/21 (90%) provided more information about the colony description/s:

- 9/19 (47%) stated that only one colony type was isolated and 10/19 (53%) stated that two colony types were isolated on the media they use



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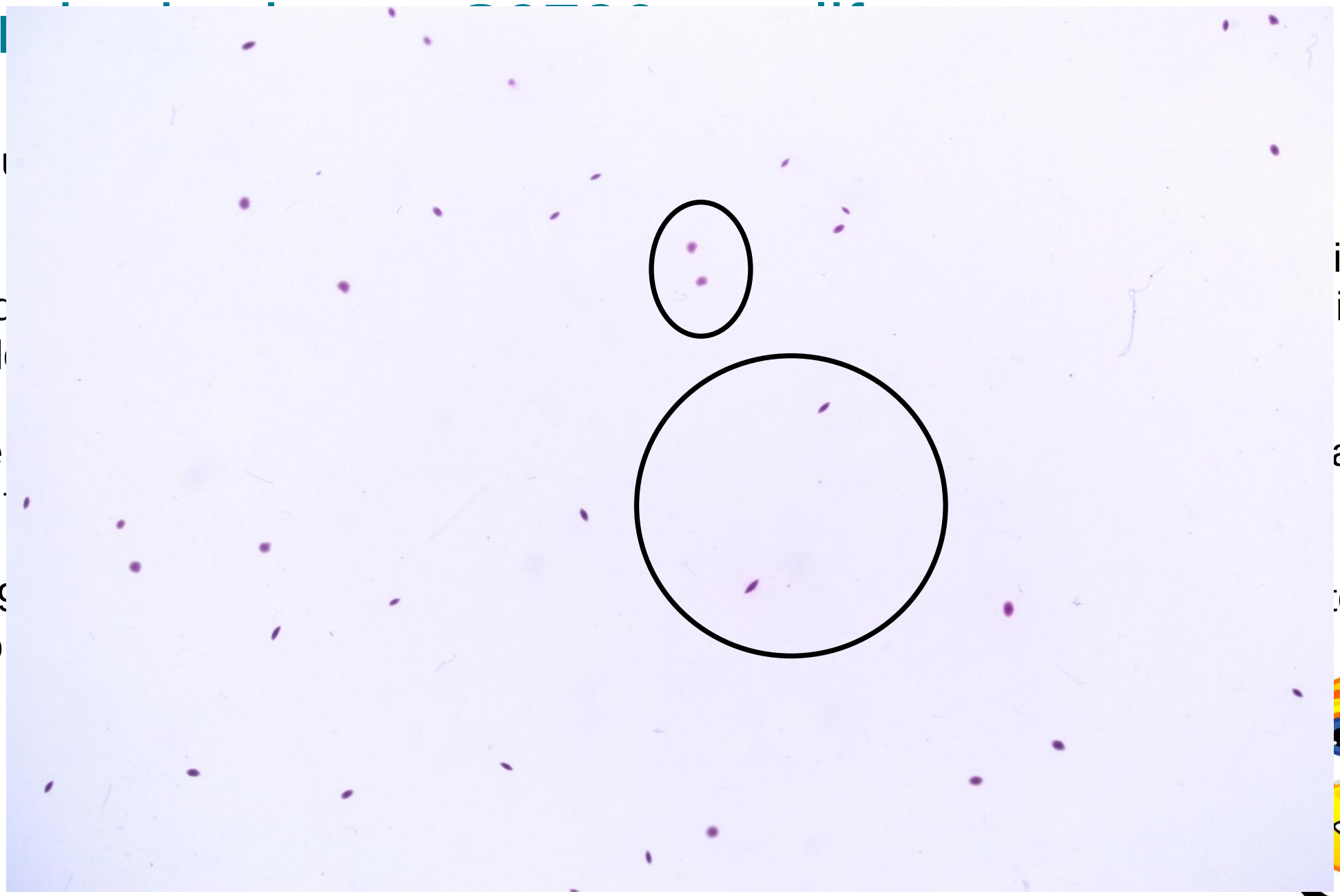

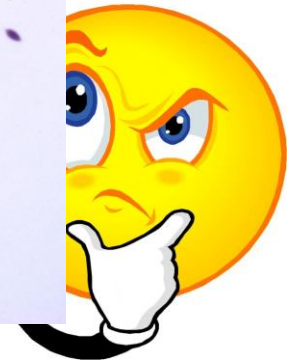

Standard scheme S0786 - coliforms

- Those reporting one colony type (n=8):
  - ❖ five stated the colonies were purple and >0.5mm
  - ❖ three stated colonies were purple and <0.5mm, two did confirmation tests and only one stated no gas was produced

- Those reporting two colony types(n=9):
  - ❖ three stated they were purple and >0.5mm
  - ➢ two did confirmation tests

  - ❖ Six stated both >0.5mm and <0.5mm purple colony types were isolated
  - ➢ four did confirmation tests
  - ➢ three did BGBB and two stated gas was produced, and one stated no gas produced

Many laboratories reported that results were presumptive and did not do further confirmation tests

Advice: if results are ’presumptive’ provide this information in the comments section when returning results

Conclusions

- ISO 4832:2006 also mentions that *'the method described in this International Standard will, on average, detect only about 90% of strains of microorganisms referred to in other publications as 'presumptive' coliforms' (e.g., certain strains of* Citrobacter, Enterobacter, Klebsiella*)'*
- This ISO was produced in 2006 – is it still relevant for the current period when methodologies have improved and changed since then. We are all aware that the methods used for identification have changed from conventional phenotypic characteristic tests to more sophisticated genotypic or rapid biochemical ones
- Is the ISO definition of 'coliform' still valid? based on newer technologies – those that do not produce gas are they considered insignificant for public health management
- There still seems to be a wide variation of testing algorithms followed – but why such a wide range of isolation and confirmation test results:
  - Is this related to the quality of media used?
  - Is this related to manufacturer differences?
  - Is this variation due to the variation on components used and the quality of these?
  - Technical competence?
  - …… and other to

So did I have a better understand of the results reported by analysing the responses from the questionnaire - Am afraid not!

Legionellae – G134B (April 2024)

Content: *Legionella micdadei (*4.9x10^5), *Legionella pneumophila* serogroup 1 (1.1x10^3) *Acinetobacter junii* (2.6x10^3) and *Pseudomonas aeruginosa* (2.2x10^4)

- 132/140 (94%) of the laboratories reported a ‘detected’ result for *Legionella* spp.
  - 24 of the laboratories failed to report that two species of legionellae were in the sample:
  - 12 failed to isolate the *L. pneumophila*
  - 12 failed to isolate the *L. micdadei*
- 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, photo is 1:10 dilution at 5 days

Legionellae – G134B (April 2024)

- It is important that laboratories undertake confirmation tests by selecting a minimum of five picks of the varying colony types for further testing
- In the FEPTU laboratory the growth characteristics of these two species were different on Glycine Vancomycin Polymyxin Cycloheximide (GVPC) agar after 7 days of incubation
- Neither species exhibited autofluorescence when viewed under ultraviolet light
- Both species did exhibit a ground glass appearance
- Other confirmation tests such as a latex agglutination or Matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry (MALDI-ToF) will assist with species identification

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

Questionnaires

Occasionally we will send out a questionnaire/s to understand (if possible):

- How participants have derived at the results they have obtained and then reported, this is especially important when there is a performance issue with an examination
- To update our knowledge on the tests done by laboratories so that we always ensure that the most common tests are included when we characterise the organism – know the behaviour and therefore can predicted the impact on PT results
- Have a better understanding of the trend in tests being carried out

So please complete the information – it’s valuable

General 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
- Process PT samples the same as other routine samples. Otherwise nothing will be learnt about your quality system
- Understand the impact on public health management when incorrect results are reported
- 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

Updates

- New website developed so will be coming off the gov.uk platform and will be launched January 2025 – it will make it easier for FEPTU to ensure that information participants need is kept up-to-date
- We are at the final stages of implementing a new software that is used to deliver the service, to be launched hopefully August 2025
- Wastewater scheme will be launched and available from April 2025
- Exploring further new scheme developments, if you have any feedback or suggestions then do let me know
- In January 2025 we will be sending out a feedback questionnaire on our services – so please complete this as it is valuable information for us to ensure services remain relevant
- FEPTU are actively working towards accrediting the services to the new version of the standard ISO/IEC 17043:2023

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Thank you for listening

Email: foodeqa@ukhsa.gov.uk