



AXIO
PROFICIENCY TESTING

Investigations into Coliform Test Results in LGC AXIO Proficiency Testing Schemes

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Definition of a coliform

Coliforms are Gram-negative rods that are non-spore-forming, oxidase-negative, facultative anaerobes. Coliforms are not defined by their taxonomy but generally include species from the four genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*.

These genera, along with *Proteus*, *Salmonella*, *Serratia*, *Shigella* and *Yersinia*, form the Enterobacteriaceae family. According to ISO 4832:2006 ⁽¹⁾ coliforms are bacteria which form characteristic colonies in crystal violet neutral red bile lactose agar (VRBL), and which in confirmation tests cause fermentation of lactose with the production of gas (under the specified test conditions).

ISO 9308-1⁽²⁾ defines coliforms as lactose positive bacteria which are oxidase-negative. Lactose positive bacteria are bacteria capable of forming colonies aerobically on a selective and differential lactose culture medium with the production of acid.

Different national regulations may also have their own definition for coliforms, for example, the United States Food and Drug Administration (FDA) defines them as Gram-negative rods which produce acid and gas as a result of lactose fermentation. ISO 4832:2006 and ISO 4831:2006 state that the definition of coliforms for each specific method “is not necessarily identical to the corresponding definitions given in other published texts.” This is true outside of ISO standards for other specific national coliform procedures.

Reasons for testing for coliforms

Coliforms may be found in a number of habitats, such as soil, aquatic environments, and the intestines of humans and animals. The presence of coliforms in food or water is therefore considered to be an indicator of cross-contamination from these habitats, and therefore a sign of poor hygiene or quality. In such circumstances the food or water may also have been contaminated by enteric pathogens. Coliforms are generally present in greater numbers and are easier to test for than pathogens, so the coliform test is used as an initial screen to assess the quality of foods and water and the possible presence of pathogens, hence the name 'indicator organisms'.

Test methods

Most methods are based on the resistance of coliforms to the presence of bile salts and their ability to ferment lactose with the production of acid and/or gas.

ISO 4832:2006 – Horizontal method for the enumeration of coliforms describes use of VRBL pour plates with typical coliform colonies appearing as purplish red colonies with a diameter of at least 0.5mm. Confirmation is carried out on atypical colonies using Brilliant Green Lactose Bile Broth (BGBB) and observing for formation of gas in a Durham tube.

Alternative culture methods include chromogenic agars, most probable number (MPN), rehydratable dried agar film, and many more.

Challenges in testing for coliforms

Coliforms are defined not by their taxonomy but rather a biochemical reaction defined in the method. Due to the differing definitions of coliforms and the many test methods available, it is possible for some organisms to give inconsistent results, leading to problems with interpretation. Some methods simply rely on the morphological appearance of the colonies on selective agar, and give a presumptive result only, whilst other methods require evidence of gas production to give a confirmed result. Each method is designed to detect or enumerate typical coliforms which make up the majority of the classification.

Unfortunately not all coliforms behave as expected under test conditions, some possess the enzyme β -galactosidase and therefore the potential to ferment lactose, but under test conditions may or may not ferment lactose to produce acid and gas. These include weak lactose fermenters and delayed lactose fermenters.

Weak and delayed lactose fermenters produce low levels of the enzymes β -galactosidase or permease, both necessary to ferment lactose. Over time in a high lactose environment, delayed lactose fermenters will more rapidly ferment the lactose to produce acid and gas. Weak and delayed fermenters may not always behave as a coliform under laboratory conditions, they can give variable results and appear negative after 24 hours using standard methods.

Observations from a Proficiency Testing (PT) Scheme

The LGC AXIO Proficiency Testing Microbiology schemes have been organised over the past 30 years in order to enable laboratories performing the microbiological analysis of food and dairy products to monitor their performance and compare it with that of their peers. LGC AXIO Proficiency Testing also aims to provide information to participants on technical issues and methodologies relating to testing of food and dairy products.

AXIO QMS Samples 16 (PT-MC-16D for the dairy matrix and PT-MC-16F for the food matrix) include tests for the enumeration of total aerobic mesophilic count, *Escherichia coli*, Enterobacteriaceae and coliforms. Results over the past few years show that the proportion of participants obtaining satisfactory z-scores for these tests is generally around 95%. However, in Round 292 distributed in August 2020, the number of participants obtaining satisfactory z-scores for coliforms was much lower than usual (with respectively 78.7% and 74.6% satisfactory results for MC-16D and MC-16F).

These MC-16D and MC-16F samples contained *Escherichia coli*, *Enterobacter aerogenes* and *Citrobacter koseri*. On investigation it was found that the *Citrobacter koseri* strain is an example of a delayed fermenter. In VRBL pour plates, the colonies appear typical of a coliform. However, confirmation using BGGB broth according to ISO 4832:2006 gives variable results. After 48 hours at 37°C no gas was observed and only 1mm of gas at 72 hours. However, when 100µl of BGGB was subbed into fresh BGGB and incubated, 2mm of gas was produced after only 24 hours. More gas is produced after subbing due to *Citrobacter koseri* adapting over time to ferment the lactose. (see Figures 1 & 2).

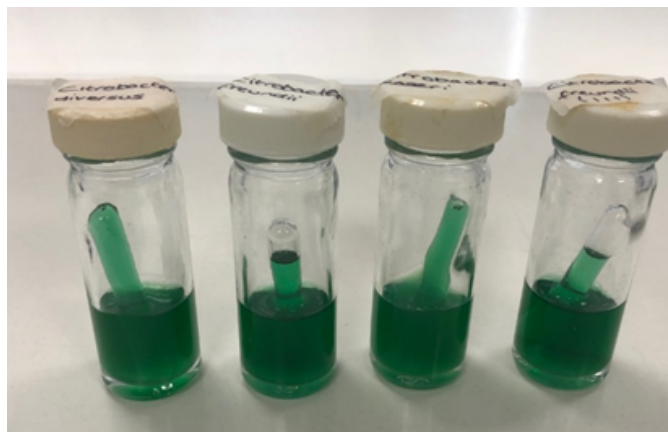


Figure 1: From left to right, cultures of strains of *C. diversus*, *C. freundii*, *C. koseri* and *C. freundii* in BGGB, showing lack of gas production by *C. diversus* and *C. koseri* after 48 hours incubation at 37°C

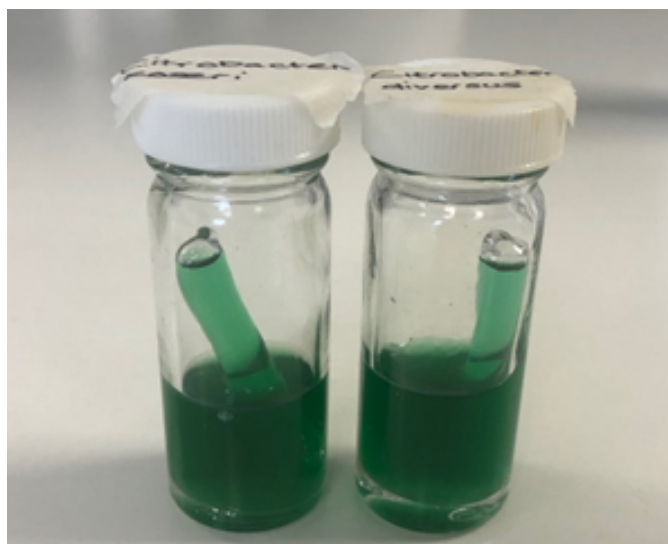


Figure 2: From left to right, cultures of the same strains of *C. koseri* and *C. diversus* from Fig 1, subbed into BGGB, and now showing gas production after a further 24 hours incubation at 37°C

Conclusion

The definition of coliforms is affected by the method used, and the interpretation of results, by individual laboratories. Coliform definitions also vary according to sample type and by national standards. As there is no taxonomic classification of coliforms, organisms classified as coliforms according to one method may not be classified or enumerated as coliforms by a different method. When participating in a global proficiency testing scheme many labs will be using different definitions of coliform and the results may therefore differ depending upon strains present in the sample. This is not a limitation of the method or laboratory but rather a result of the fact that coliforms are not defined by taxonomy. If you find that you have a result that is different to others in a round of proficiency testing in which an organism was included that does not fit your country regulations or the methods definition of coliform, you should reassess the results based on the sample contents and organisms included to explain any unsatisfactory results. In most cases, LGC AXIO Proficiency Testing addresses this by removing colour-coded assessments of such results, so as to not disadvantage laboratories applying a different definition or interpretation of coliforms compared to the majority.

In microbiology there are also almost always exceptions to the rules. Microbiological methods have been developed over time to easily test for the most common, typical microorganisms of interest in foods and water, however there are many strains which may not behave exactly as expected under the conditions of the test; these are known as atypical strains.

This means methods may not be 100% accurate all of the time, especially with less common and atypical strains, and some degree of compromise and careful interpretation of the results may be needed. For example, if an organism that could have originated from an enteric source is found in a sample, then this could indicate poor sanitary quality, regardless of whether the organism produces gas or not under the conditions of the test.

Coliforms, which may not behave as a textbook definition of a coliform under laboratory conditions, do exist in nature and could quite feasibly be present in food and water. For this reason, PT samples should contain a range of strains with different characteristics, including atypical strains, in order to reflect real life challenges. Such strains should be included infrequently to reflect their prevalence in nature, but to give participants the opportunity to challenge their methods, and to see how they perform compared to other laboratories carrying out the same tests. This may occasionally make AXIO proficiency testing samples more challenging than some other PT samples, but we strongly believe that it represents a better value to participants than providing PT samples that always contain the same strains and produce predictable results with no educational element.

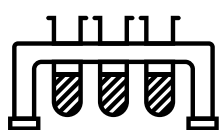
As a result of these investigations, from January 2022, for sample MC16, AXIO will offer participants the option to report their coliform results according to method of confirmation. The options will be coliforms (presumptive), coliforms (confirmed by gas) and coliforms (confirmed other than gas). This additional information should enable a better interpretation of participant results.

We welcome any comments or feedback on this issue, please contact microbiology.bury@lgcgroup.com

References

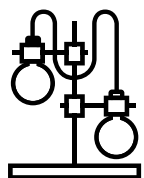
1. ISO 4832:2006: Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique
2. ISO 9308-1:2014: Water quality — Enumeration of *Escherichia coli* and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora

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