WHERE DID THAT ABERRANT *E. COLI* RESULT COME FROM?

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ABSTRACT

It is perhaps not surprising that a positive *Escherichia coli* result in a drinking-water sample from a well treated supply that usually complies may be regarded with suspicion. After all, if the treatment plant is working well and there are no other indications of a problem, and perhaps the water even has an FAC residual, how can the water really still contain *E. coli*? It is tempting to think of the result as an aberration or "false positive" caused by contamination of the sample. However, there are a number of possible explanations of such a result: sample contamination, laboratory error, false positive *E. coli*, inadequate disinfection, post-treatment contamination, and the erratic distribution of microbes in water.

The various causes of drinking-water *E. coli* transgressions are discussed as well as how they may be investigated and prevented.

KEYWORDS

Drinking-water quality; *E. coli*; false positive

1 INTRODUCTION

It is perhaps not surprising that a positive *Escherichia coli* result in a drinking-water sample from a well treated supply that usually complies with the *Drinking-water Standards for New Zealand* (DWSNZ) may be regarded with suspicion. After all, if the treatment plant is working well and there are no other indications of a problem, and perhaps the water even has an FAC residual, how can the water really still contain *E. coli*? It is tempting to think of the result as an aberration or "false positive" caused by contamination of the sample. However, there are a number of possible explanations of such a result. These are listed below, together with a discussion as to how they may arise and, where possible, how they may be investigated and prevented.

- Sample contamination
- Laboratory error
- False positive *E. coli*
- The erratic distribution of microbes in water
- Inadequate disinfection
- Infiltration of contaminated water

2 POTENTIAL CAUSES OF ABERRANT RESULTS

2.1 SAMPLE CONTAMINATION

Samples can be contaminated by a variety of mechanisms including poor sampling technique, the use of non-sterile bottles, contamination during transport and sampling in adverse conditions.

Sample bottles are a possible source of contamination that is easily avoided by ensuring that they are sterile. Sterile sample bottles are either purchased from a supplier or provided by the laboratory using the procedures and practice of bottle sterilisation that are included in the audits of all Ministry of Health-recognised laboratories involved in drinking-water compliance testing. Once sterilised, the bottles will generally be sealed and/or contain a sterility indicator. If they do not then insist that this is provided, otherwise there is no indication that the bottles are sterile or they may become mixed with non-sterile bottles. As with all sterile material, there is a shelf-life beyond which sterility cannot be assured. It is good practice to ensure that the sterile bottles are stored in a place where they are unlikely to be contaminated by dust, for example, and to rotate stock so that the shelf-life is not exceeded.

It is usual in potable water testing for the bottles to contain a small amount of sodium thiosulphate, which is essential if the water being sampled is chlorinated. This is normally added to the bottle prior to sterilisation but if it is added after the bottle is sterilised, it must be done with great care using strict aseptic technique to avoid contamination. Sterilising the sample bottles after adding the sodium thiosulphate will remove this possibility.

The presence of *E. coli* indicates faecal contamination not only in water, but in any material or on any surface. This includes the outside of the tap, hands and the places where one may place the lid of the sample container. This is why samplers must be trained in aseptic technique and be vigilant in following the correct procedures when taking samples. Flushing, cleaning and surface-sterilising the tap, keeping fingers away from the open bottle and lid during sampling, and not putting down the lid during sampling are important here. Also make sure the sterility seal is removed as failure to do so may cause contamination if it gets caught in the thread when the lid is replaced after sampling. The best way of avoiding this source of contamination is to have well trained samplers. Having well-documented sampler training procedures, regular refresher training and occasional field observation of the sampling procedure will help.

There are some adverse conditions where a sample may become contaminated. Examples are if it is raining or windy, or there is some obvious problem with the location of the sample point. When sampling in the rain it is possible that rain may get into the sample bottle directly or drip from wet clothing, for example. This can be avoided by using an indoor sampling point (if possible) or shielding the sample bottle from the rain (umbrella). Wind-blown dust may contain *E. coli*. This may be more difficult to avoid and if an indoor sample point is not available it may be wise to reschedule the sample. If this is considered to be the likely cause of contamination, it is recommended that this be investigated by exposing a number of sample bottles containing 100 mL of sterile water in the same manner as would be done during sample collection, and testing these in the laboratory. It would be wise to specify how to take samples during adverse conditions during sampler training and in the procedures manual and, if appropriate, in the public health risk management plan (PHRMP).

Samples can be contaminated during transport to the laboratory if the bottle tops are not fitted tightly. Ice used to keep the bottles chilled will eventually melt and may contaminate the samples. Using freezer blocks (slicker pads) will avoid this. Using the same container for transporting drinking-water samples and more contaminated material, such as food or wastewater samples, also adds an additional contamination risk. Using a dedicated chilly bin and cleaning it regularly will minimise this problem. These points should be covered in the procedures manual and/or PHRMP.

2.2 LABORATORY ERROR

A range of possible errors can occur in the laboratory, including mislabelling, sample contamination, use of inappropriate procedures, misinterpretation of results and transcription errors. However, all of the IANZ-accredited laboratories are assessed to ensure that adequate procedures are in place and are being applied appropriately. Laboratories are audited and reassessed regularly. While it is inevitable that the occasional mistake will occur, even in the best laboratory, this level of quality assurance has been found to be the most effective means of minimising errors. Corrective action procedures are in place to detect errors and the procedural breakdowns that cause them. This should include a procedure to re-issue reports when an error has been shown to have been made. If there are concerns that a result

may be inaccurate, it is worth raising this concern with the laboratory. A laboratory that has a genuine desire to maintain quality will investigate any concerns and report back on its findings.

If using a laboratory that is not accredited for testing *E. coli* or faecal coliforms in drinking-water, consider using another laboratory that is Ministry of Health-recognised for drinking-water compliance testing.

2.3 FALSE POSITIVE *E. COLI*

False *E. coli* positives do exist. These are nothing to do with laboratory error but are microbiological phenomena. To explain this it is first necessary to define faecal coliforms and *E. coli*.

Faecal coliforms are typically described as Gram negative, oxidase negative, facultatively anaerobic rod-shaped bacteria that ferment lactose to acid and gas and are able to grow at 44.5°C and in the presence of 1.5% bile salts. Faecal coliforms comprise a number of bacterial genera including *Escherichia* spp., a member of which is *E. coli*. In practice, however, a faecal coliform is defined by the result of the test method. It is now known that some species that give a positive faecal coliform test may originate from environmental rather than faecal sources. For this reason *E. coli* is a better indicator of faecal contamination than faecal coliforms, which is the main reason why it is the preferred bacteriological compliance test in the DWSNZ.

E. coli is a subset of faecal coliforms and is traditionally defined by its phenotypic characteristics. It is described traditionally as a Gram negative, facultatively anaerobic rod that gives positive methyl red and negative citrate and Voges-Proskauer reactions, and converts tryptophan to indole and ferments lactose to acid and gas at 44.5°C. However, the more contemporary defined substrate methods are based on an expression of β-glucuronidase activity and either ONPG hydrolysis or lactose fermentation.

In microbiological terms, a bacterium that complies with this description will be identified as an *E. coli*. However, rarely, other coliform bacteria that are closely related to *E. coli* show the same reactions. These false-positive *E. coli* are only rarely encountered in water and are unlikely to cause a major problem because they are all faecal coliforms. Nevertheless, if concerns remain that a result is a false-positive *E. coli*, ask the laboratory to identify the organism; if it proves not to be *E. coli*, then it is legitimate to change the result to *E. coli* negative and no transgression should be recorded.

NB. This only applies if *E. coli* is used as the indicator organism. If water quality is assessed using faecal coliforms then the result is a transgression because the false-positive *E. coli* is still a faecal coliform.

2.4 THE ERRATIC DISTRIBUTION OF MICROBES IN WATER

There is a widespread misconception that if *E. coli* is not detected in a 100 mL water sample then the water is free of faecal contamination. This is far from the truth. Contaminants in general are distributed unevenly in water. This is particularly so for contaminants at low concentrations such as *E. coli*. There are two different facets to this problem.

The first and most obvious cause of variation is that the sample of water taken for testing may not be representative of the water as a whole. At low *E. coli* concentrations this phenomenon may manifest as an occasional positive sample in a water supply that usually shows no *E. coli*. As a sporadic *E. coli* result may occur when the plant is apparently working properly, it is not unusual for a water supplier to think that the result is erroneous one, with sampling error often being blamed.

The second cause of variation is an uneven distribution of *E. coli* within the sample itself. This is minimised by thorough mixing of the sample in the laboratory, which is facilitated by, and the reason for, leaving some headspace in the sample bottle. However, non-homogeneity remains a problem at very low concentrations irrespective of how well the sample is mixed.

This lack of sample homogeneity can be seen by the uncertainty in the measurements (often depicted by error bars); the error bars generally become wider as the concentration decreases. This is due to the difficulty of making accurate measurements at very low concentrations as the limit of detection is approached. For *E. coli* the maximum acceptable value is close to the limit of detection. Some of the

uncertainty is caused by the test method or instrumentation, some by the operator and some by the variability of the sample. Instrument and operator variability can be controlled by training and adherence to procedures, but the variability of samples depends on their homogeneity, which decreases markedly as the concentration approaches zero. The variability of samples for chemical analyses is generally very small because even at the limit of detection, the number of target particles in the sample is very high. (For example, if the limit of detection is 0.001 mg/L for a chemical with a molecular weight of 600 Daltons, then about 10^{15} molecules per litre are required before they can be detected.) However, the limit of detection for *E. coli* is one cell per sample. This means that if the concentration of *E. coli* is just at the acceptable limit of 1 cell/100 mL and half of the 100 mL sample is tested, there is a 50% chance of the *E. coli* not being in the test portion and hence of getting a negative test result. This proportion decreases with increasing *E. coli* concentration. Even at a concentration of 5 cells/100 mL there is a 1:32 chance of getting a negative result simply because of the non-homogeneity of the sample.

It is not good practice to have a compliance limit set at the limit of detection for a test because of the increased uncertainty associated with the result, and because the test gives no warning that a problem is occurring before a transgression occurs. In the case of drinking-water *E. coli* compliance testing it would be better to enumerate *E. coli* in a volume larger than 100 mL (say a 1 L sample) and converting the result to a count per 100 mL. This will remain an issue while it is usual practice in the water industry to test 100 mL water samples.

2.5 INADEQUATE DISINFECTION

Chlorine requires sufficient contact time with the water to allow complete disinfection to occur before the water reaches the first consumer in the line. Free available chlorine (FAC) samples taken at a point where insufficient time is given for the chlorine to oxidisable material in the water may give rise to a reading that is misleadingly high. Further consumption of the chlorine with the remaining oxidisable contaminants is likely to result in there being too little FAC to adequately disinfect the water.

Where contaminant levels in the water are high, as can be shown by break-point testing, chlorine concentrations may be inadequate if organics and particles are not removed before disinfection. This can result in breakthroughs of faecal bacteria. Monitoring the incoming water is necessary to prevent this occurring.

The presence of *E. coli* in treated water that has an adequate (≥0.2 mg/L) FAC residual seems counterintuitive and is often regarded as a "false positive" *E. coli* result by some water suppliers. While this may be so, there are other plausible explanations that need to be considered.

The first, and easiest to check, is the accuracy of the FAC measurement, which equally could be in error. This can be checked by recalibrating the FAC meter (if used) or cross-checking the FAC result against another method.

The second explanation is that the FAC measurement is accurate, but the chlorine is not in a form that is able to disinfect, that is, it is at a pH that is too high. This can be checked by calculating the FAC equivalent (FACE). If the FACE is less than 0.2 mg/L, then the presence of *E. coli* is not unusual.

A third explanation is that the *E. coli* bacteria may be being protected physically from the disinfectant. This can occur if the bacterial cell is particle-bound. For example, bacteria surrounded by clay particles or those held within a biofilm can be shielded from chlorine and other disinfectants. The former will be minimised if low turbidities are maintained.

Biofilms are the layers of bacteria and other microorganisms that attach to the walls of the water pipes. They can harbour all manner of bacterial, protozoal and viral pathogens, as well as *E. coli* and harmless micro-organisms. Parts of biofilm can slough off and should the particles contain *E. coli*, they may well be protected from the residual FAC and result in a positive test result. Biofilms are an inevitable part of reticulation systems and indeed any solid/water interface. However, thick biofilms will slough off more readily than thin ones. The risk from biofilms can be controlled by scouring and maintaining a disinfectant residual. Scouring the pipes will allow safe, if temporary removal of the biofilm (and also

sediment in the pipe that can also harbour pathogens and *E. coli*). Maintaining a good residual FAC will impede biofilm growth.

It is possible that disinfection severely damages the *E. coli* cells but does not kill them. Normally this will not matter because the cells will not grow in the test medium. However, some of the *E. coli* cells that are sub-lethally damaged by ultraviolet (UV) light treatment may recover in water in the pipes by a process known as dark-repair (Chan & Killick, 1995). This may occur if the UV dose is borderline, which may be indicated if the applied UV dose is lower than usual, or the turbidity or colour of the water is higher than usual, or the flow through the UV reactor is faster than usual.

Disinfection shielding may be investigated by checking that day's turbidity, FAC, pH, UV and water pressure records. The occurrence of turbidity spikes, low FAC or UV dose, high or very low pH or water pressure fluctuations increase the likelihood of/may indicate the likelihood of inadequate disinfection or the presence of particle-bound *E. coli*.

2.6 INFILTRATION OF CONTAMINATED WATER

Even in the best treatment plants there will be the occasional time when the treatment falls below optimum. Indeed, the DWSNZ allows the occasional event when the turbidity is too high, or the disinfectant residual or UV dose is too low, without jeopardising bacteriological or protozoal compliance. At these times it is possible or even likely that *E. coli* will pass through the treatment plant and enter the reticulation system in a viable state. Check the records and if the *E. coli* was detected on a day when some aspect of the treatment was not meeting specifications, even for a short time, this may have been the cause of the transgression.

Another circumstance that may allow a slug of untreated water to pass through the pipe is a backflow event. This can allow contaminated water to enter an otherwise safe water supply and occurs when a reservoir of untreated water is hydraulically connected to the reticulation network (e.g. a stock trough where the water pipe lies below the surface of the water) and there is a drop in the water pressure. This may suck contaminated water from the trough into the reticulation where it may pass down the pipe and be a risk to other people on the same system. Check the water pressure records, and if the *E. coli* was detected on a day when the water pressure was low, this may indicate infiltration or backflow as the cause of the transgression.

A third example is where there is a break in the water main and the broken pipe is exposed to muddy and contaminated water. Contaminants are unlikely to enter the reticulation against the flow of water from the broken main. However, it will probably do so when the water is closed off to allow the repair. Of course, this risk can be mitigated by spot disinfection and subsequent flushing, as would be normal procedure following a mains break. However, a slug of contaminated water may occur if the procedures were not implemented correctly and mistakes do happen. Check the repair and maintenance records, and if the *E. coli* was detected on a day when repairs were being made to the water mains it may indicate this as the cause of the transgression.

In these circumstances it is perfectly possible for a slug of contaminated water to occur in a reticulation system that otherwise contains well treated and safe drinking-water.

3 CORRECTIVE ACTION FOLLOWING A TRANSGRESSION

The appropriate corrective action following the detection of *E. coli* in a monitoring sample is prescribed in the DWSNZ (Ministry of Health, 2008) and comprises the following steps:

- Immediate notification of the drinking water assessor (DWA) or medical officer of health (MOH)
- Resample as soon as practicable and retest using an enumerative test for *E. coli*
- Investigation to determine the source/cause of contamination and, if this can be identified, take appropriate steps to ensure that the problem does not recur. This should include retraining

samplers or amending the PHRMP, depending on whether or not this threat was covered in the PHRMP

- If another transgression occurs, or the *E. coli* concentration was ≥10/100 mL, discuss the need to notify customers of the increased risk with the DWA/MOH and take appropriate remedial action to remove the immediate risk to consumers (this may include issuing a 'boil-water' notice)
- Take daily follow-up samples until three consecutive negative *E. coli* results are achieved. Ideally this will be done to confirm the effectiveness of the corrective action. However, if the cause of the transgression is not evident, the presence of three negative clearance samples will give some degree of surety that the water is no longer contaminated.

The appropriate immediate corrective action will be obvious in some circumstances (e.g. remove the dead animal from the reservoir, or replace the broken chlorinator), but what do you do when you cannot find the cause of the problem? All too often it is assumed to be a sampling error or a laboratory error and the issue is closed after the three clearance samples return negative results. This response would be appropriate if the sampling or laboratory is shown to be at fault provided corrective action (e.g. retraining or improved sampling/laboratory procedures) is taken to prevent recurrence of those errors. However, it is not appropriate to assume sampling or laboratory error unless there is some evidence of this.

4 CONCLUSIONS

In conclusion, while it may be tempting to consider sampling error to be the cause of an aberrant *E. coli* result in a drinking-water sample that is from a well treated and usually compliant water supply, there may well be another explanation that may indicate a health risk. Unless these avenues can be ruled out, it is appropriate to take the precautionary approach, as is the norm in matters of public health, and regard the incident as a bacteriological transgression.

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