Interactive comment on "Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks" *by* L. Mezule et al.

Anonymous Referee #2

Received and published: 26 November 2012

General comments:

The manuscript by Mezule et al demonstrates the limitations of current standard methods on detecting the indicator species, E. coli, in a main supply drinking water system. The study relies on the use of DVC-FISH as an alternative and this technique clearly shows a higher detection of this indicator and the presence of VBNC E. coli. However, I have several concerns about the work, which I will detail below. In general I think further detailed discussion is required on the importance of the detection of VBNC cells in the context of whether it a) makes culture data meaningless, b) whether the wrong indicator is being chosen, c) is this important as no outbreaks have been recorded within the study timeframe? There are many studies which have recognized the presence of VBNC bacteria in such an environment and what additional information does this study

bring? I think the authors need to present stronger arguments for their findings.

In general there needs to be improvement on the writing style and use of English as several sections are confusing to read. In addition, there have been little statistical analyses carried out and rarely are data quoted in the text.

Specific comments:

Abstract:

P516, L6 "analyse the distribution of non-cultivable E. coli" – important to clarify this statement with non-cultivable but viable, there is no point in just detecting non-cultivable.

P516, L12 "cultivable, total and able to divide (viable)" – throughout the manuscript there are discrepancies on how the term "viable" is used. In this instance, it is confusing because cultivable bacteria are also able to divide and are obviously viable. More clarification of definitions is needed.

P516, L17 "almost all of the samples contained viable E. coli in the range of 1 – 50 cells per litre or cm2 which represented approx. 53% of all E. coli detected." – this sentence needs clarification, how have these 53% been detected? Does viable include those which are cultivable? And how has total E. coli (including non-viable) been calculated? There is no information on these points and although only the Abstract it makes it difficult for the reader to understand the approach.

P516, L18-20 – a comment is made that the number of viable E. coli tend to increase further from the treatment plant but no explanation offered. However, in the following sentence it is stated that it accumulates when less exposed to disinfectants – this is an obvious statement and these sentences could be condensed and combined.

P516, L23-25 – the term "viable but not cultivable" is used for the first time, this should be used in the earlier definitions.

General point – what is the clear conclusion of the work, what new information does it reveal? If the focus is to be how DVC-FISH is an improved method, this should be stated in the abstract.

Introduction:

P517, L17 – the authors state that it remains disputable whether the VBNC state exists but there is now an extensive amount of more recent literature demonstrating the existence.

P517, L20-25 – comments are made regarding the retention of pathogenicity but no mention of infectivity studies on VBNC bacteria in mice, rabbits, embryos and amoebae (amongst others) is made.

P518, L7 – the authors refer to detecting total, cultivable and VBNC (detected as DVC-FISH) cells, however, care must be taken to acknowledge that DVC-FISH will detect cultivable plus VBNC. The number of VBNC can be calculated by subtracting cultivable values from DVC-FISH results.

Methods:

In general it is noted that some sections of the methods are given in detail (e.g. composition of hybridization buffer) and other sections are very vague.

P518, L20 –a reference from 2010 is given related to water quality but no acknowledgement is made that this can change within a short time frame and within a network and so the referenced data may not be applicable.

P519, L5 – the biofilms were tested after 2 weeks, this does not seem a very long period to allow biofilm development and can this really be considered to be comparable to long term biofilm which will have built up over years? Some discussion is needed in the later sections.

P519, L12 – the water concentration step is noted as taking between 12 and 24 hrs, are

the samples kept at a constant temperature through this time period? Is there evidence to show that differences in concentration time do not affect results?

P519, L14 – the recovery efficiency is stated as 81±33% , this means in some cases it was as low as 48% or as high as 114%, I feel a comment on this needs to be made as it can clearly affect results considerably.

P519, L18 – the volume of the sample should be stated.

P519, L25-29 – more detail is required to be able to understand the ATP method.

P520, L8-10 – although following a standard method, it would be useful for the reader to have more information on the medium used, incubation conditions, etc.

P520, L13 – no details are given on the type of FISH, i.e. DNA or PNA probe? No reference provided for earlier work comparing to molecular methods.

P521, L2 – following hybridization the filters were washed in distilled water only, usually a washing step using a washing buffer for a certain period of time (e.g. 30 mins) is used to ensure no non-specific binding. Could the authors make reference to this change in method or provide evidence of validation?

P521, L3 – provide details on microscope used.

P521, L4 – how was a detection limit of 4 and 6 cells calculated – was the entire filter scanned for labelled E. coli? Normally the multiplication resulting from using a low number of fields of view results in a higher detection limit.

Results & Discussion:

P522, L13 – it is stated that sampling has been carried out from water source to tap but nowhere in the text is there mention of testing from a tap – could this be clarified.

P522, L26/27 – a lowering of detection limits is mentioned but no details are provided comparing detection limits so it is impossible for the reader to assess this. Detection

limits (including calculation) are needed for each technique.

P522, L28-30 – it is noted that more cells were found in the spring and winter months (it is not known whether this was statistically significant) suggesting no influence of warmer external temperatures but it is also stated that the water temperature remains the same regardless of the time of year so why would an effect of outside temperature even be investigated?

P523, L17-18 – "existence of naturalized E. coli, although no pathogenicity was proven" – do the authors mean "naturally occurring E. coli"? And they do not show any pathogenicity why should we be concerned?

P523, L25 – using the terminology of "about million cells" should be clarified, at least provided in scientific notation 106 cells.

P523, L26 until end of paragraph – not clear what point is being made, could the authors explain. Also a R2 value of 0.8869 is quite low.

P524, L17 "role of biofilm community on pathogen accumulation" – this should be explained in more detail as it is not clear in the text how the linear correlation relates to this.

In general, there also needs to be a discussion on the limitations of the DVC-FISH method – is it practical for routine screening, etc? And more discussion on whether existing measures are sufficient as there has been no outbreaks?

Conclusions:

The conclusions do not appear to add any new information to the existing knowledge. More emphasis should be made on the effectiveness of the method and a comment on whether existing measures are sufficient.