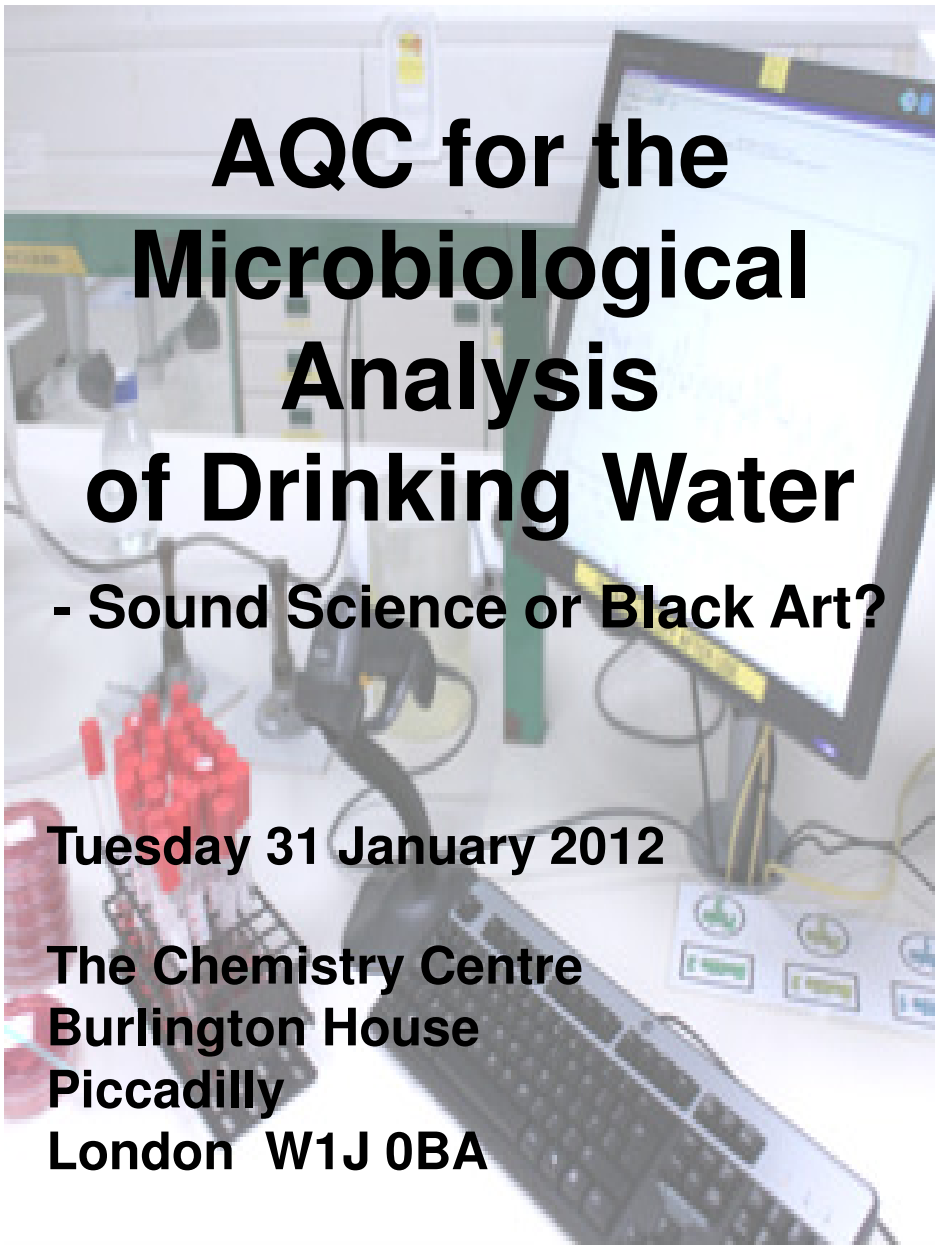
The Royal Society of Chemistry
Water Science Forum



AQC for the Microbiological Analysis of Drinking Water

- Sound Science or Black Art?

Tuesday 31 January 2012

The Chemistry Centre
Burlington House
Piccadilly
London W1J 0BA

Outcomes from the Breakout Session and the Open Forum

RSC | Advancing the Chemical Sciences
Water Science Forum

The Breakout Session

The delegates were divided into six groups and each group was asked to address two questions that were relevant to the aims of the conference, as follows:

Question 1 – *In terms of analytical quality control, can we agree on what is definitely in control, probably in control (a grey area), probably out of control (a grey area), and definitely out of control? And how much grey is permissible?*

All six groups covered this question to some degree. Their responses were very similar and are summarised below:

- Unlike chemical analysis, there is usually no opportunity to repeat or re-run a microbiological analysis, or to take fresh fit for purpose sub-samples – therefore there is a need for total quality management and a "get it right first time approach".

- There is a potential element of risk associated with every stage of a microbiological analysis so any failure of any part of that analysis (e.g. catastrophic equipment failure such as incubator temperatures going out of limits; failure of a negative control; failure of a positive control; failure of a blank sample) has to be investigated fully and the impact assessed.

- A clear distinction must be drawn between issues relating to analytical quality samples and issues that may genuinely be associated with all test results (e.g. deterioration of the media, inferior product use or damaged selectivity during media preparation). The latter may well be picked up by trends in quality control results and need to be recognised and rectified as early as possible.

- Drinking water samples could themselves form part of the quality control investigation process, given that coliform organisms should in theory be absent. (e.g. where a blank or negative control fails but positive controls are as expected and all the samples are negative, or if a significant number of unrelated samples that would normally be expected to be negative suddenly turn out to be all positive.)

- The skills and judgement of the analyst form a vital part of any investigation and subsequent assessment. This means that human factors can play an important role in the final outcome and good training and robust procedures must form part of the process.

- Rather than trying to define grey areas of judgement, there is a need for a robust system of investigation. This is an area where a decision tree might be a useful tool in providing a formal route between acceptance and rejection of the results.

- Historically the water industry and the regulator have used the SCA series of Blue Books on Microbiology of Drinking Water as best practice and there is already a very successful DWI- Water Laboratory Liaison Network Group. But this does not necessarily focus on microbiological analysis, so is there a need for a specific DWI Liaison Group looking at issues relating to microbiological analysis? Or should more emphasis be placed on microbiological analysis within the existing Liaison Network Group, given that the continuing development of molecular techniques means that chemists and microbiologists will need to work more closely together in the future?

Question 2 – *How do you decide on the source of the reference material to use and what are the comparative merits of using Vitroids®, Lenticules ® or making controls from your own cultures?*

Two groups looked at this question. Seven participants in one group used Lenticules®, four used Vitroids®, and four prepared their own controls from cultures. This was for a various applications, some qualitative, and with the reasons being 'horses for courses'. The participants in the other group all used Lenticules® or Vitroids®, with none preparing their own controls. The responses of the two groups are summarised below:

- Developing competition between suppliers is seen as useful in terms of range/type of products available and also the cost of products available. One participant gave the example of preferential use of Vitroids® for Legionella.

- Overall Vitroids® were found to be more robust than Lenticules®. However laboratories could consider using a mixture of the two but this would probably be too bespoke to be economically viable.

- Factors influencing choice included:
    - Traceability;
    - Stability;
    - Availability;
    - Cost;
    - Ease of use;
    - Level and range of count; and
    - Method recovery

- Sometimes the choice was made because the strain included provided a desirable challenge to the method. This also applied to the specifics of sample preparation and subsequent behaviour in the test (e.g. typical behaviour in response to heat treatment for Legionella)

- Some participants preferred to use their own quality controls because some organisms, such as Aeromonas, did not perform well from the reference materials available. Others preferred to use their own cocktail of reference organisms to approximate a real sample, which was then used in tests for several target organisms.

- Is there a need for more collaboration between the water industry and the regulators to ensure that the sources of all quality control material conformed with UKAS requirements?

Question 3 - *Should the positive control always be prepared from a specified culture (e.g. E. coli NCTC 9001)? If so, what would be a realistic target level?*

One group looked at this question and participants were in favour of using specified cultures provided that a suitable range was used to test the system. A system of regular review would have to be built in, which would also address the issue of 'how many and how often'.

Question 4 - *Are external microbiological proficiency schemes providing what is really required? Do they sufficiently 'test' the positive and negative aspects of the analysis and is their move to reporting z-scores technically or statistically appropriate for microbiology?*

One group looked at this question and responded as follows:

- The value of external proficiency schemes is in providing additional quality information, though it is questionable as to what degree spiking levels are set and whether they are appropriate.

- External schemes provide good variation in terms of positive and negative readings, but they are not so good in respect of matrix variation. Samples need to be representative of the real world.

- Turn round times for reporting on a given distribution are important to the end users and it was noted that they are improving.

- Most external schemes are honest when there are any problems and this enables laboratories to evaluate genuine issues.

- The current LEAP Emergency Scheme involves the analysis of chemical unknowns and no microbiological species. Would anything be gained from extending this scheme include "relevant" micro-organisms?

- The group did not express strong views in relation to the reporting z-scores in external schemes. However this remains a highly contentious issue with several experts considering them to be inappropriate in this context.

Question 5 - *How would you explain to a chemist the setting up of microbiological QC limits, frequency of review and dealing with issues such as trend analysis and step changes? If everyone is moving towards Shewhart charts, what is the value/role of doing duplicate split samples to create guidance charts?*

One group, which happened to contain a number of chemists, looked at this question and responded as follows:

- There were similarities with setting up control limits in chemistry. However the main value of a Shewhart chart is as a guide to day to day control.
- Vitroids® and Lenticules ® provided good data for trend analysis and if these were used, there was no need for duplicate split samples.
- In the absence of Shewhart charts, duplicate split samples were essential.
- It should be noted that the two approaches have some fundamental differences in what is being tested (For example, duplicate split samples look at repeatability and not just the count)

Question 6 - *In chemical AQC you can derive a batch to batch target mean to look at long term performance. How should an equivalent target mean be evaluated in microbiology? How many Lenticules®/Vitroids® and over what period?*

One group, which happened to contain a number of chemists, looked at this question and responded as follows:

- This is an area where microbiology is different from chemistry. In chemistry you are looking at the distribution of a very large number of molecules in solution, whereas in microbiology you are looking at the dispersion of a very much smaller number of organisms in a sample.
- The first factor is batch to batch variation in the reference product supplied – even if relatively small. Initially guidance charts would be set around the suppliers' expectations. Thereafter they would be reviewed for any trends or deterioration and also reviewed retrospectively to identify actual performance over the whole batch.
- Batches can be overlapped to compare the performance of a new batch against the previous one. Limits for a new batch would tend to be referenced to the suppliers' expectation and as laboratories tend to generate between 2 and 4 AQC results per day, there is no difficulty generating data. Limits for a new batch could also be set on the basis of experience, for example, the analysis of 50 AQC samples from each new batch. However this would be a costly process.
- Performance would be reviewed periodically for each batch in use and retrospectively on a batch to batch basis to give a longer term picture. Performance would also be compared against the suppliers' expectations.

- Limits around the target tend to be wider for AQC results obtained during normal application when compared with those performed on an initial batch by a single more experienced analyst and calculated specifically to derive the target mean.

- There has to be a good deal of confidence in AQC results and hard and fast rules are less appropriate than in chemistry. A decision to reject a whole set of tests would, therefore, only be made on the basis of a considerable body of evidence.

Question 7 - *Looking at microbiological AQC in the context of wider quality assurance, what are the best ways of estimating the level of uncertainty of measurement?*

One group looked at this question and concluded that human factors probably contributed highly in estimating uncertainty of measurement. Reference was made to BS 8496:2007 Water quality. Enumeration of micro-organisms in water samples. Guidance on the estimation of variation of results with particular reference to the contribution of uncertainty of measurement.

The Open Forum

This took place after the afternoon presentations and was intended to provide delegates with an opportunity to raise any outstanding issues. Points raised included:

- In terms of quality assurance there has to be some commonality between chemistry and microbiology. However this does not apply to specific definitions such as limits of detection. For example, a result may be reported as not detected in 100ml, as per the Drinking Water Regulations, but the result might be different for a larger volume of sample. The same applies to levels of uncertainty of measurement.

- BS EN/ISO/IEC 17025:2005 (General Requirements for the Competence of Testing and Calibration Laboratories) allows laboratories to determine the limits of detection and also has a section on estimating levels of uncertainty of measurement. It was therefore agreed that harmonisation of definitions was essential for good practice.

- Although there is a requirement to be able to quote uncertainties of measurement, there appears to be relatively little interest in the subject. Even so the question was raised on how to respond to a customer requesting such information and it was agreed that the guidance given in BS8496 was the best way forward.

- However in case of a single sample with a result of less than the limit of detection, the uncertainty of measurement is theoretically infinity – but, if required, an estimate of the uncertainty of measurement for the method could be assessed statistically from AQC data. Therefore it is better to build up a picture over time of uncertainty of the test results rather than looking specifically at uncertainly of measurement. Such a pragmatic approach is totally dependent on a good record of AQC results.

- Concerns were raised that a result remained valid even when investigations into a microbiological failure showed that the sample was not representative of the water in supply (e.g. caused by the poor condition of the sample tap). The DWI view was that the laboratory was in control of the analysis up to the point that the final result was released and it was then up to the company to investigate the failure and submit a report of its investigation. However, the result would still remain on record even if the investigations showed that the sample as analysed was unrepresentative.
  The counter argument is that there are levels of uncertainty associated with all stages of the process from sampling through to final reporting and there

is a potential risk at each stage that the sample may become compromised. The investigations therefore must be sufficiently robust to demonstrate the validity, or otherwise, of the reported result and, although the Regulations require the result to remain on record, a suitable caveat should be attached to explain why it is considered to be unrepresentative.

- Finally Dr Sue Passmore endorsed the need for all microbiologists in the audience to consider engaging more fully in the work of the relevant BS/ISO Committees involved in developing new or updated microbiological standards for drinking water. This could take the form of direct involvement or simply providing feedback to consultation documents.
  Likewise there was a plea for more water microbiologists to get involved with the activities of SCA Working Group 2.
  Such contributions are vital in the development of standards and further improving good practice.