



Microbial Source Tracking (MST) – survey design and data interpretation

Operational instruction 985_10

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What's this document about?

This document provides guidance on the use of Microbial Source Tracking (MST) field survey techniques to assist with the determination of the sources of microbial contamination. Its use is primarily targeted at bathing waters and shellfish waters.



Document details

Who does this apply to?

Staff in:

- Area Environmental Management (EM) Teams;
- Area Analysis & Reporting Teams (A & R);
- Regional Data, Information and Assessment Teams (DIA);
- Regional Water Quality Planning Teams (RWQP).



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Feedback

Contact for queries

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Background

MST identifies different sources When investigating faecal pollution it helps to be able to identify the different sources (e.g. sewage, cattle, or birds) which are contributing microbial contamination at compliance sites such as bathing waters and shellfish waters.

MST provides supporting information Microbial Source Tracking (MST) is a developing tool which can provide supporting information in the identification of the sources.

Bacteroidetes method

Bacteroidetes used as surrogate for FIOs Ideally we would use a method which could distinguish directly between different strains of the Faecal Indicator Organisms (FIOs) of interest (e.g. faecal coliforms, faecal streptococci) to determine and quantify the sources. Unfortunately this is not currently possible on a routine basis at a reasonable cost. Instead an anaerobic bacteria, Bacteroidetes is used as a surrogate for these FIOs.

What are bacteroidetes? Bacteroidetes are abundant gut bacteria excreted by most animal species of concern. Genetic markers, derived from small segments of the DNA sequence of Bacteroidetes, are specific to the type of animal in whose gut the Bacteroidetes reproduce. These are counted as gene copy numbers using a molecular microbiological technique called qPCR (quantitative Polymerase Chain Reaction).

What the laboratory reports When a sample of river or sea water is submitted for analysis, the laboratory reports a 'gene copy number (GC)' as a numerical value for each of:

- human specific genetic marker;
- ruminant specific genetic marker; and
- general marker which indexes the total Bacteroidetes population in the sample (i.e. potentially from humans, ruminants, and other sources).

The general markers make up the bulk of the total markers, but we have to rely on the much smaller numbers of human and ruminant specific markers to identify the sources.

Interpretation of MST data

Qualitative interpretation

The results can indicate whether the Bacteroidetes present were predominantly derived from say human or ruminant sources, in a qualitative (i.e. presence/absence) manner. Thus, if a sample is positive for human, but not ruminant Bacteroidetes, it is possible to state that pollution from human sources was significant in that sample at that time.

Quantitative interpretation

A direct quantitative assessment is more contentious, and at best the results should be considered semi-quantitative. To explain how this works, a hypothetical sample result is discussed below in a question and answer session.

Questions and answers

Sample result

Suppose the sample analysis shows the following results:

- General Marker 200,000 GC;
 - Human Marker 3,000 GC;
 - Ruminant Marker 2,000 GC.
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Would it be justified to assume that there are 5,000 specific markers of which 3,000 come from humans: hence the contribution of Bacteroidetes to this water sample is 60% human and 40% ruminant?

The laboratory validation data, and the UKAS quality control procedures, provide confidence that there were 5000 specific markers (human + ruminant) in that sample. Using the data to infer (quantitative) sources is more questionable. The answer is that it is probably not appropriate to assume 60% human, 40% ruminant in that water body. We don't have good empirical data to prove that the ratio of the specific marker GC number to the total species-specific excretion of Bacteroidetes is uniform between humans and ruminants. Thus, quantitative estimates may prove contentious, and could cause problems, if used as the 'sole evidence' to underpin regulatory enforcement and/or expenditure decisions.

Can the qualitative and/or quantitative estimates of human and ruminant Bacteroidetes species loadings at a compliance point, which are suggested by MST results, be used to infer the relative species contribution of the faecal indicator compliance parameters for bathing and shellfish harvesting waters?

Given that Bacteroidetes species and the FIO compliance indicators are excreted by all warm blooded animals, there is the expectation that some correspondence between the two types of gut bacteria would be expected. Data from a substantial number of bathing water samples do show a significant relationship, but the data are noisy. Therefore, caution is needed in this interpretation for two reasons.

Firstly, we know that the FIOs are reduced dramatically through disinfection processes, such as UV treatment of sewage, and they subsequently reduce in the environment due to natural processes such as sunlight irradiance and microbial predation. The MST test uses molecular methods to identify specific sequences of DNA which seem to pass through UV disinfection with no apparent reduction in GC number.

Thus, where sewage is subjected to UV disinfection the results may well be misleading and a dominant human MST signal may be simply reflecting the presence of sewage-derived FIOs which have been rendered non-viable through UV disinfection.

In general, there is little information on the differences between FIOs and bacteroidetes in terms of their fate as they are transported through different environments. The possibility that they behave quite differently means that caution is required in interpretation, particularly when considering the impact of sources some distance from the sampling location.

Secondly, given the noise in the relationship, it is not possible to make precise predictions of FIO concentrations from Bacteroidetes concentrations. There are also differences in the relationships between Bacteroidetes and coliforms; Bacteroidetes and enterococci (and between coliforms and enterococci).

During 2009 artificial samples in known proportions were made of mixtures of sewage and animal waste to investigate how closely MST represented the proportions of FIOs. These trials demonstrated a weak, but significant correlation between concentration of FIO and Bacteroidetes. The correlation is better between Bacteroidetes and coliforms than Bacteroidetes and streptococci (enterococci). This difference is not surprising as coliforms and streptococci themselves frequently show poor correlation.

How should the MST test be used to provide operational insight into possible FIO loadings at regulated water?

MST provides a useful exploratory tool but it is not a silver bullet which can provide a credible regulatory evidence-base from a small number of samples. Treat it as a 'guide' not as 'proof' of source. MST data have, in most situations, matched the expectations of local staff. And when MST data have challenged our assumptions, they have proven to be correct several times. Therefore the data should be given serious consideration.

Rivers

The available data suggest that rivers and streams can and do experience very short term switches in dominant MST and FIO loadings, particularly through rainfall events where CSOs and intermittent agricultural sources can impact at different stages in a hydrograph event. A sequence of MST samples collected through characteristic events (say 30 per event) can be very informative in understanding this pattern. But, clearly, interpretation of a single or very few, samples from a river would provide little credible data to underpin an Environment Agency interpretation of upstream inputs impacting from a catchment source.

Bathing and/or shellfish harvesting waters

Generally, the MST results give a good indication of the sources of faecal contamination in a large body of water. However, intensive sampling (hourly) over several days has produced some evidence of 'flipping' between human and ruminant dominance in past studies. This flipping appears to be real and not an artefact of the MST method. Thus, again, a reliable interpretation is dependent on acquisition and analysis of a sufficient number of samples to use some mean assessment as the human and/or ruminant contribution. In particular staff making such interpretation should be aware of the sewage treatment systems in the vicinity of the sampling point and the inability of MST to detect a reduction in FIOs inactivated by UV treatment. Where membrane filtration is the disinfection process used, MST should reflect correctly the reduction in FIOs. Currently we have insufficient evidence to indicate the position where chemical methods (chlorine/hypochlorite, peroxy-acetic or peroxy-formic acid) are used

Is it sensible to interpret a human signal as indicative of a large sewage plant and a ruminant signal as indicative of rural farm-derived diffuse pollution?

Generally this is what you might expect but the key thing is to know your catchment, i.e.:

- sewage effluent will at times give a strong ruminant signal where there is, for example, agriculturally influenced surface drainage in the combined sewers and/or where an abattoir is connected to the sewerage system;
 - rural farming areas can produce a strong human signal where domestic septic tanks and soak-aways impact on a river or adjacent coastal water; and
 - CSOs spilling into a riverine source impacting on a coastal bathing water might present a situation where human sources are indicated but the pollution is related to the riverine input.
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Mitochondrial DNA

Alternative approach

The bacteroidetes method was developed to distinguish human from ruminant (cattle and sheep) sources. Feedback from initial users identified a wish to be able to detect contamination from dogs and coastal birds. Neither of these species discharges unique bacteroidetes in sufficient numbers for that method to be applied, and an alternative approach using mitochondrial DNA is used.

What are mitochondria ?

Mitochondria are membrane enclosed organelles (sub-units within cells) which are the source of energy in most eukaryotic cells (cells containing a nucleus).

Dog, bird, human

The method used for MST distinguishes dog, bird, and human mitochondria. Detecting the human signal is necessary as bird mitochondria may be present in the human signal through eating poultry.

Independent assessment

Usefully the human signal gives a totally independent assessment to that derived from bacteroidetes.

Non-quantitative

The mitochondrial DNA method is non-quantitative. However a dog or bird signal is a useful indication that these species may be a significant source warranting further investigation.

Conclusions

Conclusions

- MST gives accurate information on the source of bacteroidetes in the sample.
 - If sufficient samples are analysed, MST usually gives a good indication of the source of FIOs in the catchment. Source contributions may change rapidly during, for example, rainfall events, and inference from a few samples collected over a short time period is therefore inappropriate. Although MST will give an indication of the relative importance of the sources it does not give accurate quantification of the sources.
 - Where UV disinfection is employed, we believe that MST will normally over-estimate the sewage component, but we have yet to have any hard proof of this.
 - A decision on the source of microbial contamination should never be made on the basis of single (or few) samples which do not characterise a representative range of input flows and environmental conditions encountered by the receiving water.
 - In a number of instances where MST has given non-intuitive results, further investigation has shown MST to be correct. This comment should not be taken to imply that MST will always give the correct answer.
 - MST should never be the sole basis of a management decision.
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