

# science summary



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## Microbial Source-Tracking Project Science Summary SC070003/SS

In order to manage faecal pollution in our bathing waters, we need information to help us locate the sources of such pollution. A new report by the Environment Agency outlines a routine procedure which can be used by agencies and local authorities to trace the origins of faecal bacteria found in bathing waters.

The aim of this project was to develop a toolbox of methods to help environmental managers trace the sources of pollution in rivers and bathing waters. Stage 1 of the project involved training staff and developing a simple routine microbial source-tracking service, based on quantitative polymerase chain reaction (Q-PCR), to distinguish faecal pollution from human and farm animal sources for the 2007 bathing water season. Stage 2 was to develop and test new markers to allow further discrimination of possible sources of faecal pollution from birds, dogs and humans.

The service developed in Stage 1 was (and continues to be) offered in a staged process. Samples for microbial source-tracking (MST) were collected in parallel to standard bacteriological samples. A measured volume was then filtered, and the filter stored in a stable state (frozen) for a small fee. This allowed users of the service to be provided with the bacterial count data, from which to decide how to proceed – discard, continue to store, or process for DNA extraction and measurement of the three marker sequences for general, human and ruminant animals. The ability to store the samples for a small charge was welcomed by all users in the project as a way of ensuring maximum value from their budgets.

For Stage 2, a variety of possible markers for birds and dogs was investigated, along with an alternative marker for humans. All markers were screened against individual faecal samples from known host animals to confirm specificity and sensitivity. The best performing candidate markers were characterised and the information gained from each evaluated. These will be incorporated into the routine service in due course.

An application for UKAS accreditation was made for the initial tests in December 2007, and it is anticipated that

accreditation for the laboratory procedures will be awarded during 2008. Applications for accreditation for the new markers will likewise be made to UKAS.

The report concludes that *Bacteroidetes* methods have the key advantage in being able to provide data on the relative contribution of each marker bacteria from each animal host group to the total faecal bacteria. However, the method is not without drawbacks, and other methods may be developed in the future to verify the source-tracking data.

The development of novel markers for source-tracking using mitochondrial DNA in this report demonstrated exceptional specificity, and may be potentially useful for detecting pollution from birds. However, this potential must be balanced against several drawbacks. Firstly, the link between the faecal bacteria causing a regulatory failure, and the DNA marker used to try and explain that failure, is as yet unknown, and is further compounded when using mitochondrial DNA than when using *Bacteroidetes* DNA. Secondly, the presence of bird DNA from the human diet is readily detected using these assays, and thus the assay requires assessment of at least two markers (bird and human), increasing costs.

Nevertheless, the approach outlined in this report remains the first potential marker for the detection of pollution from birds, and is applicable across the UK. The ability to test for DNA from birds (as class Aves) has advantages in that separate assays are not required for each bird species and geographical region. As the tests are used further, the database upon which correlations between the bird marker and other indicators (such as faecal streptococci) will increase and can thus be explored.

This summary relates to information from Science Project SC070003 reported in detail in the following output(s):

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