SUMMARY COMMENTS AND STATUS OF HORIZONTAL DS 3. *E. COLI* AND *SALMONELLA*

RECOMMENDATIONS FROM THE STEERING COMMITTEE

Mr T Simonart, Work Package Leader in substitution of Mrs De Roubin (transferred to the USA), highlighted that there were three different methods under CEN standardisation process for determining the presence of *E. coli* and *Salmonella*. In order to evaluate their performances on each kind of sludges, soils and biowastes matrices, experimental work was needed.
The Steering Committee would like to see the development of methods for rapid analyses, if technically and legally possible (the issue of patented systems was mentioned). However, it was highlighted that commercial methods could not be standardised. **It was agreed to go ahead with the experimental work**.

SUMMARY OF COMMENTS

HORIZONTAL DESK STUDY 3. FEASIBILITY OF HORIZONTAL STANDARDS FOR *ESCHERICHIA COLI* AND *SALMONELLA* IN SLUDGES, SOILS, SOIL IMPROVERS, GROWING MEDIA, AND BIOWASTES.

Authors: Richard SHEPHERD
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The report has evaluated the current existing methods available for the detection and enumeration of *Escherichia coli* and *Salmonella* spp. with a view to implementing horizontal standardisation.

The formulation of a horizontal standard to cover sludge, soil, soil improvers, growing media, and biowaste is considered feasible, however there are several key areas which require resolution through ruggedness and interlaboratory testing.

The final report will stress the following matrices sludge, soil, soil improvers, growing media, and biowaste which are the specifically mentioned matrices in the six draft CEN microbiological standards. These should be submitted for CD enquiry very shortly after the recent CEN TC308/WG1/TG5 meeting in London.

A key issue highlighted from the report is that it is much more difficult to assess the effectiveness and recovery rates achieved during sample preparation than it is during the

detection stage. The first stage, which involves separating the bacteria from the solids present in the matrix is much harder to evaluate. This should be addressed in the proposed FP 6 study.

With regard to the detection stage of the methods it is essential to have a stable reference material in order to obtain a microbiological standard to check the effectiveness of the methods. The MICROCRM conference recently held in Lille discussed the issue of commercially available microbiological reference materials. Also reference materials if prepared in a solid dry matrix simulating the matrix being tested could in theory be used for crudely assessing the effectiveness of the sample preparation stage. Some work is being carried out in the UK on this aspect. In our view, it is essential to have a Europe-wide validation of the methods. It is of no use to test the robustness of the methods on a small scale just with a few prestigious non-routine research institute type laboratories.

There has to be an improvement in the level and quality of the performance data attached to the methods. The advent of a proficiency scheme for sludge, soil, soil improvers, growing media, and biowaste matrices should be actively encouraged. The setting up of a Europe wide scheme would make it possible to assess the extent of variation in testing between laboratories.

It would be useful to arrange a large-scale round-robin exercise that would compare the commercially developed methods against the conventional six draft CEN methods. The main advantage of the commercial methods is that they do involve as many variables as the CEN standards and should be more robust. If it is found that they show significantly better performance data we should recommend them as an acceptable alternative approach. The more rapid analysis turnround time to a confirmed result for most of these commercial methods is another advantage.

The rate of throughput for the methods varies between the rapid methods developed commercially by IDEXX, Matrix Micro-science, and Merck which are able to generate confirmed results within 24 hours. The draft CEN standards use more traditional methods which can take up to three to four days to provide a confirmed result.

The draft sludge directive quotes a *Salmonella spp*. detection limit of absence of any viable *Salmonella* spp. in 50g of received material. Of the three methods proposed, the presence/absence method has the best potential limit of detection of ~1 viable organism in 10g of received material. The membrane filtration methods is limited to thin sludges with less than 10 –20 % dry solids content, it has to be questioned whether or not the limit of detection specified in the directive is achievable for *Salmonella spp.*

The membrane filtration Salmonella method is primarily concerned with log reduction and only involves a temperature resuscitation step unlike the other two draft *Salmonella* methods.

An interesting thought is to question why the draft limit for sludge testing is absence in 50g and yet the limit in food microbiology is absence in 25g?

In order to take the methods through to horizontal standards it is essential that thorough testing of all the proposed the methods is carried out. The FP6 project will hold the key to a lot of the questions still to be answered.

SUMMARY OF COMMENTS

HORIZONTAL DESK STUDY 3b. rapid methods for detection of *E. coli* (including *E. coli O157*) and *Salmonella*

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The report has evaluated the current existing methods available for the rapid detection and enumeration of *Escherichia coli* and *Salmonella* spp. with a view to implementing horizontal standardisation. The main methods that are available for the detection and enumeration of *E. coli* (including *E. coli* O157) and *Salmonella* have been developed largely for analysis of food and water and can be broadly divided into four groups. Proprietary Quantitray® technology, equivalent to the 5-tube most probable number (MPN) technique, employing disposable plastic trays for enumeration of *E. coli* and *Salmonella*. Immunological, involving a short or overnight pre-enrichment of the target organism followed by specific detection of cellular antigen in either a lateral flow device or following immunomagnetic capture. Molecular, involving PCR amplification of target DNA sequences from low numbers of cells, or preferably following a short pre-enrichment of the organism to amplify numbers and demonstrate viability prior to molecular detection. Physico-chemical, involving techniques such as measurement of impedance changes during enrichment and growth in appropriate media. The merits of each were described, in relation to their suitability for use with sludge, soil and biowastes. In particular, there are two requirements demanded in the draft standards: i) A quantitative requirement to detect less than so many *E. coli* per weight of material, as well as being able to demonstrate process efficiency through a 6-log drop for endogenous *E. coli* and a spike organism such as *S. senftenberg* in some draft standards. ii). A sensitive presence/absence requirement to show absence of *Salmonella* per weight of material

The first requirement may appear to be met for *E. coli* using the Colilert® or Colilert-18® system in under 24 hours, and this should be a priority for inter-laboratory evaluation in the next phase of Project Horizontal. The requirement for quantification of *Salmonella* is more difficult because there is not an equivalent of the Colilert® technology available for these pathogens. For the second requirement, it is still not apparent that the molecular and immunological methods available for direct detection are suitable for detection of *Salmonella* in sludge, soil and biowastes at less than 1 cell per g. Perhaps this situation could be improved by using immunological clean-up recovery (e.g. IMS and recirculating IMS: PATHATRIX) followed by a sensitive PCR procedure not affected by an inhibitory background. Consequently, the second requirement might have

to be met in under 24 hours if there is a reliable enrichment procedure for *Salmonella*. The problem is that many methods using pre-enrichment/enrichment media specify at least 48 hours, partly because of the slow growth rates of some of the serotypes, particularly if they have been environmentally stressed and become sub-lethally damaged. Further work will be required to try and accelerate the enrichment process and select more optimal growth conditions. For now, there are various procedures which reliable enrich *Salmonella* in 48 hours, including the draft CEN presence/absence method for sludge and commercial systems such as the S.P.R.I.N.T pre-enrichment/enrichment (Oxoid). Once grown out, the *Salmonella* can be reliably detected in minutes using lateral flow devices such as the GLISA Singlepath (Merck). These are much faster than PCR techniques, simpler to use in a busy laboratory and arguably cheaper. It is recommended that the Singlepath or other similar devices be evaluated in Project Horizontal. At least this approach will give a result in 48 hours, possibly 24 hours, unlike present methods taking up to 4 or 5 days.

Consequently, we consider it feasible to formulate horizontal standards to cover rapid analysis of sludge, soil, soil improvers, growing media, and biowaste. However these are complex matrices compared to what the methods were originally designed for i.e. food and water. Consequently, there are several essential stages of method development that must ensure robustness and reproducibility in interlaboratory testing, utilising sample pre-treatment to overcome potential matrix problems of recovery and interfence with the detection method. None of the methods described have been extensively evaluated for sewage sludge, soils or biowastes. As such, there is an urgent need for their modification and evaluation as part of the next phase of the Project Horizontal.

We should like to thank the reviewers for their useful comments which will be incorporated into this Desk study and the overall presentation of the Hygienic Parameters Studies 1-7.

The general comments made relate to the whole series of reports in the WP3 Hygienic Parameters. Several reviewers asked that a general introduction to the WP3 be included at the start, in particular describing why Desk study 3A: *E. coli* and *Salmonella* methods is different from Desk study 3B: Rapid *E. coli* and *Salmonella* methods. We did this description in the 3B introduction but the reviewers have asked that something like this should be at the start of the whole set of studies in the WP. Also, 3A contained some quite detailed information on rapid *E. coli* methods that several reviewers suggested should only be detailed in our 3B report.