SUMMARY COMMENTS AND STATUS OF

HORIZONTAL DS 4. *CLOSTRIDIUM & ENTEROCOCCI*

RECOMMENDATIONS FROM THE STEERING COMMITTEE

The Project Leader indicated that the desk study received positive comments. The Steering Committee **agreed to go ahead with the experimental work** need for determining the technical validity of the proposed methods.

SUMMARY OF COMMENTS

HORIZONTAL DESK STUDY 4. Feasibility of horizontal standard methods for detection of *Clostridium perfringens* and *Enterococci*

Author: C. William KEEVIL (University of Southampton)

The report has evaluated the current existing methods available for the detection and enumeration of *Clostridium perfringens* and enterococci with a view to implementing horizontal standardisation. The main methods used for the detection and enumeration of *Clostridium* and *Enterococcus* spp. have been developed largely for analysis of food and water and can be broadly divided into three groups. Quantification of colonies on agar media; most probable number (MPN) quantification in indicator broth using conventional test tube technology; and proprietary Quantitray® technology equivalent to the 5-tube MPN technique employing disposable plastic trays for enumeration of enterococci. The merits of each were described.

Inevitably, the method requirement will be based on regulatory considerations. Should there be demonstrable process control procedures involving, for example, demonstrating a 6 $\log_{10}$-decrease on treatment or should there be merely a requirement for absence in 10, 25 or 50 g wet weight of sample?

We are not aware of any requirements for a strictly quantitative method being required at this time, which suggests that multiple tube MPN or presence/absence methods should be satisfactory. Given that assays should be specific, sensitive and preferably cheap (including labour costs), then two strategies seem appropriate.

The first involves further development of the Enterolert system due to its relatively high, semi-quantitative, precision; convenience, without requiring a lot of equipment or staff time; and speed, producing results conveniently in 24 hours. The system has been trialled

and validated for various low turbidity samples and is now beginning to be used more for waste waters.

The second involves development of overnight enrichment culture techniques followed by one of two technologies, either:
disposable lateral flow devices, similar to the Singlepath technology. A convenient overnight enrichment culture of the target organism can be prepared and then confirmed in only a few minutes serologically. The challenge for this test will be to find an appropriate antibody (specific and sensitive for, say, the Lancefield's group D antigen) capable of bulk production for large demand in the market place.
Molecular labelling, using for example DNA oligonucleotide probes linked to biotin (e.g. Aureon, Vienna). Once these hybridise to the overnight culture of target cells, they can be labelled with streptavidin-linked to an enzyme producing fluorescence or light for sensitive detection.

Either of these could be made semi-quantitative, by running serial dilutions, for example in microtitre plate format, confirming positive wells using the detection technology and applying look-up tables to calculate the MPN. However, this seems pointless, given the potential for the Enterolert system to do this already and probably more cheaply. In reality, the lateral flow devices come into their own for presence/ absence determination e.g. no enterococci in 10 or 50 g wet weight sample. This approach would overcome problems with having to disperse and filter a complex, fibrous matrix such as soil or biowaste for quantitative analysis whilst giving a specific identification of the live organism without further tedious, expensive confirmation tests.

Both of these approaches should also be applicable to Clostridia, where initial easy growth of the target organisms in an anaerobic environment is an important prerequisite.

Consequently, we consider it feasible to formulate a horizontal standard to cover 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. 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 of the series of Desk studies.