

METHOD STATEMENT

Determinand:

Cryptosporidium and *Giardia*

Matrix:

Raw, potable, effluents, wastewaters, gravels.

Principle of Method:

The samples (or eluate – see comments below) are filtered onto a filter module and eluted using a manual or automated Wash station. The eluate is then filtered through a membrane and further concentrated using centrifugation. The resultant pellet containing any *Cryptosporidium* or *Giardia* is captured on magnetic beads via a technique called Immuno Magnetic Separation (IMS) and the isolates dissociated and the resultant solution spotted onto a microscope slide and dried. This is then stained using a specific Monoclonal Antibody FITC conjugated stain ready for microscopy. The Slides prepared are visualised under an epi-fluorescence microscope at x200 magnification by a DWI approved microscopist and any presumptive *Cryptosporidium* or *Giardia* are confirmed at x 1000 magnification where size, shape, staining characteristics, internal contents and morphology is determined.

Sampling and Sample Preparation:

Sample are filtered through Filta-Max[®] filters or Filta-Max xpress[®] filters.

Interferences:

Samples must not be allowed to freeze during or after transport as this may result in the production of ice crystals within the oocyst or cyst. This can change the buoyant density and/or lead to disruption of organelles within the oocyst or cyst, which may interfere with the detection, and/or identification of oocysts or cysts if present in the sample.

Performance of Method:

Limit of Detection: 1 oocyst in the volume analysed

Normal Reporting Level: 0 oocysts/10L

References:

Water Supply (Water Quality) Regulations 2000, SI No. 3184 England and the Water Supply (Water Quality) Regulations 2001, SI No. 3911 (W.323) Wales.

Environment Agency - The Microbiology of Drinking Water (2009) Part 14 – Methods for the Isolation, Identification and Enumeration of *Cryptosporidium* oocysts and *Giardia* cysts.

Comments:

The gravel samples are washed with 0.1% Tween.

