METHOD STATEMENT

Determinand:
Cryptosporidium and *Giardia*

Matrix:
Raw and potable water

Principle of Method:
The sample is filtered onto a filter module and eluted using an automated wash station. The eluate is then concentrated using centrifugation. The resultant pellet containing any *Cryptosporidium* or *Giardia* is captured on magnetic beads via a technique called Immunomagnetic Separation (IMS), 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 and any presumptive *Cryptosporidium* or *Giardia* are confirmed at x 1000 magnification where size, shape, staining characteristics, internal contents, and morphology are determined.

Sampling and Sample Preparation:
Samples are taken and provided to the laboratory as Filta-Max® filters, Filta-Max xpress® filters or a grab sample (to be filtered by the laboratory).

Filters or grab samples may be transported to the laboratory at ambient temperature. Samples that cannot be processed immediately should be stored in the range 5 ± 3 °C in the dark and should not be allowed to freeze.

Analysis should begin as soon as possible after sampling and preferably on the day of receipt within the laboratory. For samples analysed according to the DWTS accredited UK reference method (The Microbiology of Drinking Water (2010) Part 14), this should be within 48 hours. For samples analysed according to the ISO 15553:2006 reference method, this should be within 4 days.

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 (2010) Part 14 – Methods for the Isolation, Identification and Enumeration of *Cryptosporidium* oocysts and *Giardia* cysts.

BS ISO 15553:2006, Water Quality – Isolation and Identification of Cryptosporidium oocysts and Giardia cysts from water.