METHOD STATEMENT

Determinand:
Cryptosporidium Enumeration

Matrix:
Sample Type: ground waters, surface waters, drinking waters, swimming pool waters, and associated matrices

Principle of Method:
A high pressure mercury vapour lamp in a special housing is used to generate a broad spectrum of light including ultra-violet and blue light. This light is filtered by band-pass filters to produce a specific wavelength which will give maximum excitation for the fluorochromes being used to stain the (oo)cysts. Fluorescein isothiocyanate (FITC) bound to specific antibodies is used to stain the walls of (oo)cysts, whilst 4' 6-diamidino-2-phenylindole (DAPI) is used to stain nucleic material. The stained material is examined using a light wavelength suitable for FITC (filters for 450-490nm excitation and 560nm emission) and a low power magnification, for example the 20x objective. The whole of the material on each slide should be examined for characteristic (oo)cyst features. Where these are observed the slide should be re-examined using a high power magnification, for example 100x water or oil immersion objective.
Each fluorescing body should be carefully assessed for its size, shape, stain and morphological characteristics. It is more advantageous to use the microscope in the 20x and 100x objective, both in fluid immersion mode, to enable switching from lower magnification to higher magnification during microscopic examination to be carried out more easily. Each fluorescing body should then be examined, firstly, using light of a suitable wavelength for DAPI (UV filter with an excitation of 350nm and emission of >450nm) to demonstrate characteristic nucleic material, and secondly, by Nomarski differential interference contrast (DIC) microscopy to identify any internal structures within the fluorescent body. Only when the whole slide has been examined can the number of confirmed bodies be reported as a final count.
Prior to use, it is important that the microscope is optimised for Köhler illumination and Nomarski DIC microscopy (see operating procedure Microscopy – Use of Microscopes). The transfer of particulate material or beads to a slide during the dissociation stage of the IMS procedure can partially or totally obscure (oo)cysts, and can interfere with the confirmation of internal structures using DIC microscopy. The inclusion of air bubbles
during the mounting of the slide can also affect DIC microscopy. The presence of fluorescent particulate material in the fluorescent stain can make correct microscopic examination of (oo)cysts difficult. This is because large amounts of non-specific fluorescing material may be transferred to the slide during the staining procedure.

Interferences:
Residual sediment and non-target organisms may be transferred to the sample slide during the Immunomagnetic Separation (IMS) procedure. These can mask and partially obscure oocysts and cysts present on the sample slide.

Performance of Method:

Range of Application:
Normal Reporting Level: 0 oocysts/10L

Limit of Detection,
Estimated every 5 years.
Limit of detection = the number of organisms known to be added in the inocula at the end point dilution.

Uncertainty of measurement:
Recalculated every 6 months

References:

Environment Agency - The Microbiology of Drinking Water (2009) Part 14 – Methods for the Isolation, Identification and Enumeration of Cryptosporidium oocysts and Giardia cysts.
Water Supply (Water Quality) Regulations 2000, SI No. 3184 Engalnd and the Water Supply (Water Quality) Regulations 2001, SI No. 3911 (W.323) Wales.