EXECUTIVE SUMMARY

This study was initiated primarily to determine whether or not the analytical procedure associated with the Regulatory monitoring of Cryptosporidium could be modified to effect the simultaneous isolation of Giardia cysts without compromising the recovery of Cryptosporidium oocysts.

The experimental protocol involved spiking a rig containing either FiltaMax™ or Envirochek™ filters with oocysts/cysts, followed by a flow of approximately 1000 litres of treated water. This procedure was effected firstly in the laboratory (Phases 1 and 2) and secondly at five Field Trials sites (Water Treatment Works) (Phase 4) owned by Severn Trent Water. The recovery efficacy was determined for each spiking/filtration event and for both oocysts and cysts, to produce a series of recovery statistics for analysis.

In the Laboratory Trials, the median recovery rate for Cryptosporidium oocysts was maintained within the target value of ≥30% for spike concentrations of 1000, 100 and 10 oocysts/cysts for both FiltaMax™ and Envirochek™ filters. Thus, the co-isolation of Giardia cysts along with Cryptosporidium oocysts using the combination (Dynal GC Combo) immunoisolation and staining procedures did not compromise performance requirements for Regulatory monitoring.

For all of the spike concentrations and for both FiltaMax™ and Envirochek™ filters, the median recovery rates for Giardia cysts were significantly lower than the equivalent for Cryptosporidium oocysts and, except for the highest spike concentrations (FiltaMax™: 1000cysts), fell below 30%. The median recovery rate of 0%, achieved for Giardia cysts following inoculation of the 10 oocyst/cyst spike, suggests that using this experimental protocol/analytical procedure, 10 is above the detection limit for Cryptosporidium oocysts and at or below that for Giardia cysts for both FiltaMax™ and Envirochek™ filters. If it is assumed that the mechanical entrapment (filtration) efficiency is equivalent for oocysts/cysts, the apparent loss of Giardia cysts may be attributed to either the destruction of the cysts during subsequent stages of sample processing or the inability to detect them. The microscopic appearance of cysts was very variable in terms of both shape and staining intensity, and the differentiation between fluorescing Giardia-shaped debris and Giardia cysts was often difficult because of the volume of debris and the poor quality of staining.

The Envirochek™ filter performed consistently better than FiltaMax™ in terms of recovery of Cryptosporidium oocysts (100, 10 spikes). However, this was not the case for Giardia cysts, where median recovery rates for 100 spike inocula were equivalent, but less than 30% for both filter types.

Recovery data generated during the Field Trials, using spikes of 100 oocysts/cysts, were disappointing when compared with those obtained in the laboratory-based investigation. Giardia cysts were recovered from only 12% of spiked filters and in very low numbers (FiliaMax™/Envirochek™ combined) compared with 100% in the laboratory. Although this effect was less
pronounced for *Cryptosporidium*, recovery statistics for oocysts were also compromised as the investigation moved from the laboratory to the field. Whereas laboratory experiments yielded a 100% positivity rate (Filtamax™/Envirocheck™ combined) with 89% of samples attaining the target minimum recovery of 30%, only 95% of samples in the field were positive with only 27% having a recovery rate of 30% or more.

The chemical profile of treated/filtered water may be a significant factor in determining recovery rates for both cysts and oocysts. For example, in terms of chlorine concentration, a CT value of 36mg.min⁻¹ (residual chlorine 0.03mg.l⁻¹) attained for the water filtered in the laboratory, would effect a 90% reduction in numbers of spiked *Giardia* cysts. This effect could be amplified up to 20 times at the Water Treatment Works where residual chlorine concentrations may be as high as 0.5mg.l⁻¹ and contact times up to 48 hours (if the transportation time is taken into consideration). Unlike *Cryptosporidium* oocysts, *Giardia* cysts are very susceptible to chlorine, even at low concentrations. The failure to detect cysts may be due either to the physical destruction of the structural integrity of the cysts during sampling, transportation and/or processing, or chemical modification of cell surface epitopes to prevent binding of labelled antibodies. This may be of some concern in terms of detection of *Giardia* cysts during both risk assessment studies and during a waterborne outbreak, when it may prove difficult to identify potential sources of contamination. In addition, this effect, however mediated, may potentially impact on the results generated during Regulatory *Cryptosporidium* monitoring programmes, where oocysts may be present but remain undetected.