Thames Water Plc
Research and Technology

Evaluation of *Easy*Seed™ *Cryptosporidium* oocyst spike doses within a DWI approved laboratory

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Aim of Trial

To compare recoveries of a commercially produced *Cryptosporidium* spike dose with a flow generated spike dose prepared by Thames Water within a Drinking Water Inspectorate (DWI) approved laboratory.

Methods

The product ***Easy*Seed™** (batch: ES-C100-64) was provided by Biotechnology frontiers. It comprises of tubes containing a specific number of oocysts which have been counted using a flow cytometer. These spike doses were tested and recovered using the Standard Operating Protocol (SOP) for the monitoring of *Cryptosporidium* oocysts in treated water supplies (Anon 1999). ***Easy*Seed**™ was compared to a flow prepared *Cryptosporidium* spike that was prepared within the authorised laboratory.

The trial took place over a two week period with either one or two of each spike type being tested each day. This spacing out of the trial allowed any possible variation between days to be assessed.

Preparation and filtering of spike doses

I. A new filter module was inserted into a positive control filtration housing.
II. The prepared filtration housing was attached in the correct orientation to sample tubing, passing through a peristaltic pump set to provide a sample filtration rate of 1L per minute.
III. A 10L barrel containing a magnetic follower was filled to 10L (± 100ml) with deionised water and placed onto a magnetic stirrer.
IV. A *Cryptosporidium* spike dose produced using flow cytometry was vortexed for no less than 2 minutes and then carefully poured into the 10L barrel.
V. 20mls (± 1ml) of deionised water was added to the empty tube and vortexed for no less than 1 minute. This rinse was carefully poured into the 10L barrel.
VI. Section 5 was repeated TWICE. After the final rinse to ensure that there were no bubbles or froth left in the tube or the lid, it was necessary to wash the tube and lid with an excess of deionised water.
VII. The 10L spiked sample was pumped through the filtration apparatus.
VIII. 1L of deionised water was added to the barrel, the sample tubing was removed and the lid of the barrel was replaced. The contents of the barrel were shaken vigorously, the barrel was put back on the magnetic stirrer and the sample tube was replaced.
IX. The 1L deionised water barrel rinse was pumped through the filtration apparatus.
X. The filter housing was disconnected from the peristaltic pump/ sample tubing and the filter module was then ready for processing.

Methods for sample processing

The method used is detailed in the Standard Operating Protocol to satisfy the water supply regulations 1999 (Anon. 1999), this methodology includes concentration steps using the genera Filta-Max system combined with a centrifugation step and purification using immunomagnetic separation (IMS). The sample slides were stained according to the SOP using a monoclonal anti-*Cryptosporidium* antibody (FitC) and 4',6-Diamidino-2-phenylindole (DAPI). To remove the staining solutions from the slide surface, an aspirator vacuum source was used set at minimum suction (<5cm Hg vacuum), with a micro-pipette tip attached. The tip was never allowed to come into contact with the well surface, limiting the possibility of sucking oocysts off the slide.
The flow sorted control spikes were prepared using a Becton Dickinson FACScalibur flow cytometer, following the Thames Water Laboratory procedure protocol. This procedure has been accepted for routine use within the DWI approved laboratory.
Two full method negative controls were carried out using the same apparatus as used for the spiked samples to ensure that washing procedures for the equipment were sufficient and that the equipment was oocyst-free prior to sample concentration.

Results

The results obtained are presented in Table 1, each pair of ***Easy*Seed™** and flow control samples were processed on the same day, with the same reagent batches. They were also stained and enumerated together either on the same day as they were processed or the following day.

Table 1 The recoveries of *Cryptosporidium* oocysts detected from ***Easy*Seed™** and flow control spikes, using the full DWI approved method

Sample No.	***Easy*Seed™**	Flow controls	Negative controls
1	64	61	0
2	66	65	0
3	74	54	-
4	68	57	-
5	57	64	-
6	58	67	-
7	70	54	-
8	54	73	-
9	59	62	-
10	70	63	-
Mean	64	62	0
Standard Deviation	6.683313	5.906682	0
Range	54 - 74	54 - 73	0

Conclusions

- The ***Easy*Seed™** spikes produced similar recoveries of *Cryptosporidium* oocysts to the flow sorted control spikes used within the authorised laboratory.

- The oocysts observed appeared well stained with both FITC and DAPI, and none of the oocysts were clumped.

References

Anon. (1999) Standard Operating Protocol for the sampling of *Cryptosporidium* oocysts in treated water supplies to satisfy Water Supply (Water Quality) (Amendment) Regulations 1999, SI 1524.