

1. Background

It is recommended that laboratories testing water for the presence of *Cryptosporidium* maintain an adequate system of quality control and quality assurance. An essential part of a quality system is to regularly monitor recovery by analysing water samples that have been seeded with known numbers of oocysts¹. EasySeed™ simplifies and standardises these quality control tests.

The preparation of precisely enumerated seed doses is extremely difficult due to inherent inefficiencies of counting techniques² and the fact that oocysts and commonly have an uneven distribution in suspension³. When counting the number of oocysts and within replicates of a suspension, very high coefficients of variation are often obtained³. Consequently, when using aliquots of the stock suspension to seed a quality control sample it is not possible to determine the exact number of oocysts added. This makes it extremely difficult to interpret recovery data with the required level of accuracy.

The difficulties encountered when enumerating oocysts within seed doses can be completely overcome by using flow cytometry to prepare seed material³.

The use of live oocysts in a routine laboratory is hazardous. Inactivated oocysts are preferable. However, oocysts that have been inactivated by chemical or heat treatment may have undergone changes in their surface

chemistry that can affect recovery efficiencies. Gamma irradiated oocysts are an excellent alternative.

EasySeed™ combines flow cytometric aliquoting, gamma irradiation sterilisation and stringent quality control to provide a convenient and reliable seed preparation.

2. Product description

EasySeed™ is a 5mL test-tube containing one hundred *Cryptosporidium* oocysts in approximately 1mL of saline solution. EasySeed™ counts are extremely accurate. Flow cytometry is used to accurately dispense oocysts. The standard deviation of the number of oocysts in each tube is less than 2.5.

EasySeed™ tubes are sealed and sterilised by exposing each batch to a specified dose of gamma irradiation. This ensures that EasySeed™ is stable for 4 months and that the organisms in the tube are inactivated.

3. Product quality control information

EasySeed™ are supplied with a Certificate of Analysis. This document clearly shows the counts of *Cryptosporidium* made from a number of EasySeed™ tubes in the batch. A mean and standard deviation for the batch is displayed for easy reference.

Each batch of EasySeed™ is quality controlled by testing 3% of batches <1000. For batches >1000 the QC batch size is 3 X 3√n.

Tubes spaced evenly throughout the batch are selected, stained with FITC labelled antibodies and enumerated using flow cytometry as described previously². The total number of oocysts in each tube is recorded as well as the number of full oocysts, the number of empty oocysts and the number of partially empty oocysts.

DAPI staining is performed and the percentage of oocysts that stain with DAPI is recorded.

Each tube of EasySeed™ is individually weighed.

Each batch of EasySeed™ must meet the following standards:

Standard deviation	<2.5
% of oocysts/cysts that are full	>95%
% of oocysts/cysts that stain with DAPI	>95%
Variation in weight	<0.10g

4. How to perform accurate enumeration of *Cryptosporidium* within EasySeed™

The numbers of *Cryptosporidium* within an EasySeed™ tube can be accurately checked by collecting the oocysts onto a membrane, staining the membrane and then enumerating the oocysts using microscopy. A protocol for performing this analysis is available on the Internet at www.btfbio.com. The protocol must be closely followed to obtain accurate results. Technical assistance is available via e-mail at: contact@btfbio.com.

5. Assessing *Cryptosporidium* recoveries using EasySeed™

Composition of the QC sample

A water sample appropriate for use as a quality control sample should be collected or prepared. It is important that the QC sample is known to contain no *Cryptosporidium*. The presence of *Cryptosporidium* in the QC sample may affect the integrity of any laboratory QA/QC system. The QC sample may be reagent grade water, treated drinking water, untreated surface water or another water type that is similar to the samples routinely analysed by the laboratory. The QC sample should be treated exactly as standard test samples at all times during and after preparation.

Variation of recoveries between water types

The recovery of oocysts will vary from sample to sample depending on the type of water sample. For most *Cryptosporidium* test methods recoveries are lower for more turbid samples. If the laboratory is analysing a range of water types then it is important that recoveries for each water type are assessed.

Seeding the QC sample

- 1 Add 2ml of 0.05% (v/v) Tween 20 to the tube.
- 2 Replace cap and shake vigorously.
- 3 Remove cap and pour *EasySeed*™ into sample.
- 4 Add 3ml of reagent grade water to the tube.
- 5 Replace cap and shake vigorously.
- 6 Remove cap and pour *EasySeed*™ into sample.
- 7 Repeat steps 4, 5 and 6.

Sample analysis

Analyse the QC sample as per the laboratory standard operating procedure. The QC sample should be analysed using exactly the same methodology as that used to process samples. The reagents used to perform the QC test should be of the same batch as those used to perform the corresponding tests.

Record the number of *Cryptosporidium* detected in the sample, and compare to the number of *Cryptosporidium* contained in the *EasySeed*™ (shown on the accompanying Certificate of Analysis). The recovery achieved by the QC test (expressed as a percentage) is the number of cysts or oocysts detected, divided by the *EasySeed*™ number, multiplied by 100.

In equation form:
Recovery (%) = $\frac{\text{oocysts detected}}{\text{EasySeed}^{\text{TM}} \text{ number}} \times 100$

6. Technical assistance

Technical assistance with the use of *EasySeed*™ or with other *Cryptosporidium* testing problems is provided by BTF at:

E-mail: contact@btfbio.com

Fax: + 61 2 8877 9101

7. References

1. Standard Operating Protocols for the Monitoring of *Cryptosporidium* Oocysts in Treated Water Supplies to Satisfy Water Supply (Water Quality) Amendment Regulations 1999 SI No 1524. UK Drinking Water Inspectorate.
2. Bennett JW. Gauci MR. Le Moenic S. Schaefer FW. Lindquist HDA. A comparison of enumeration techniques for *Cryptosporidium* parvum oocysts. *Journal of Parasitology*. 85(6):1165-1168, 1999.
3. Reynolds DT. Slade RB. Sykes NJ. Jonas A. Fricker CR. Detection of *Cryptosporidium* oocysts in water: techniques for generating precise recovery data. *Journal of Applied Microbiology*. 87(6):804-813, 1999.

Warranty

The products are warranted to the original purchaser only to conform to the quantity and contents stated on the product labels for the duration of the stated shelf life. BTF's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at BTF's expense, of any products which shall be defective in manufacture, and which shall be returned to BTF, transportation prepaid, or at BTF's option, refund of the purchase price.

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Issue: 5
Date: 1st April 2004

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