

Cryptosporidium & Giardia

Tips on improving *Cryptosporidium & Giardia* recoveries.

1. Take care when washing slides

Cryptosporidium and *Giardia* can be washed off slides during the staining process. To avoid this carefully remove liquid from the slide by gentle aspiration.

2. Slide drying can affect *Giardia* recoveries

Inefficient neutralization of the acid used in the IMS procedure can damage the cell surface of the *Giardia* cysts, causing variable staining of *Giardia*. This can result in *Giardia* being missed during the microscopy stage. Drying the samples rapidly or keeping the sample cold ensures that the surface of the *Giardia* cysts is not damaged.

To optimize this process, ensure that:

- Once the sample has been neutralized, dry the slide rapidly at 37°C (maximum drying time of one hour) and then stain immediately.
- If there will be a delay in staining after IMS neutralization then dry the slide rapidly at 37°C and then place in a refrigerator overnight. Alternatively, dry the slide (uncovered) in a standard household refrigerator overnight at 4°C. Slides dried in a cool room may not dry as some cool rooms maintain a humid environment.

3. IMS dissociation

With some water samples a high proportion of the *Cryptosporidium* or *Giardia* can be lost in the IMS beads and are not recovered during the acid dissociation. Performing a second acid dissociation can improve recoveries significantly.

With the IMS dissociation, a second dissociation can be useful. For samples that give low recoveries the waste supernatants from the IMS process can be collected, allowing re-processing of those wastes if recovery acceptance criteria are not met. The supernatant can be collected in the 50mL centrifuge tube that the sample was concentrated in prior to IMS, this helps avoid labelling mix ups and the like. This is particularly useful when using ColorSeed™ - you can tell which samples have low recovery and run them through a second round of IMS. A lot of materials which interfere with IMS are removed in the first round of IMS, so second round recoveries can be dramatically higher.

4. Rinse sample containers and test tubes

Rinse everything that the samples comes into contact with using 0.05% tween 20 or tween 80. Add the rinsings to the sample.

5. Use EasySeed™ or ColorSeed™ for spiking

The use of EasySeed™ and ColorSeed™ gives you a reliable bench mark for measuring the performance of your test method.

EasySeed™ and ColorSeed™ are manufactured using a gentle cell sorting process that does not damage the *Cryptosporidium* and *Giardia*. Controls produced with other flow cytometry methods damages the *Cryptosporidium* or *Giardia* resulting in poor morphology and poor recoveries.

Remove the EasySeed™ or ColorSeed™ from the fridge and place on the bench 15 mins before using to take the chill off the tubes.