

Product Note

Frequently Asked Questions - US EPA Method 1622/1623

1. I am getting poor *Cryptosporidium* staining. How do I fix this problem?

Poor staining of *Cryptosporidium* oocysts occurs when the antibody does not adequately fix to the surface of the oocysts. To optimize *Cryptosporidium* staining with EasyStain™, ensure that you:

- Use the *Fixing Buffer* that is included in the EasyStain™ kit.
- Use the *Fixing Buffer* directly from the refrigerator or place on ice whilst using. The *Fixing Buffer* must be applied to the slide **cold**.
- Leave the *Fixing Buffer* on the slide for at least **2 minutes** prior to washing off.
- Only use the *Mounting Medium* included in the EasyStain™ kit. The *Mounting Medium* has been optimized to reduce fading of the EasyStain™ antibody.

2. I am getting poor *Giardia* staining. How do I fix this problem?

Inefficient neutralization of the acid used in the IMS procedure can damage the cell surface of the *Giardia* cysts, causing variable staining of *Giardia*. 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:
 - Dry the slide rapidly at 37°C and then place in a refrigerator overnight. Stain the following day.
 - 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. Do I need to include the methanol fixation step?

Methanol fixation assists permeabilization of *Cryptosporidium* and *Giardia* prior to DAPI staining. Some customers have reported that the methanol fixation step improves DAPI staining. Others report *Cryptosporidium* and *Giardia* appearing crumpled under the microscope and a minor detrimental effect on FITC staining.

- The methanol fixation step of the EasyStain™ protocol can be omitted at the discretion of the laboratory.