Cryptosporidium Staining (Auramine)

(for *In Vitro* Diagnostic use only)

PRODUCT CODE: SEE MATERIALS PROVIDED

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INTENDED USE

For use in detecting oocysts of *Cryptosporidium* species in prepared faecal smears from clinical specimens.

SUMMARY AND EXPLANATION

Cryptosporidium was first identified in 1976 and is one of the most common waterborne diseases found worldwide. Modifications of the acid-fast staining procedure can be used to identify *Cryptosporidium* species; this method uses a high concentration of phenol to facilitate penetration of the fluorescent dye into the cell wall of oocysts.

PRINCIPLE OF THE TEST

Both the outer and inner walls of *Cryptosporia* oocysts, as well as internal structures, are stained by the auramine O dye; the phenol component accelerates penetration through oocyst walls. The fluorescence is retained in the cell wall after decolourisation. A counterstain is then used to darken the background and other organisms, which emphasises the fluorescent oocysts.

MATERIALS PROVIDED

Ready to use Stains and Differentiators:

-	PL.7072	Cryptosporidium Fixative	500ml
-	PL.7033/100	Auramine Phenol	100ml
-	PL.7033/25	Auramine Phenol	250ml
-	PL.7033	Auramine Phenol	500ml
-	PL.7034	Auramine Phenol	1000ml
-	PL.7035	Auramine Phenol	2000ml
-	PL.7077	Cryptosporidium Differentiator (3% HCl)	500ml
-	PL.7039/100	Potassium Permanganate	100ml
-	PL.7039/25	Potassium Permanganate	250ml
-	PL.7039	Potassium Permanganate	500ml
-	PL.7040	Potassium Permanganate	1000ml

Per 100ml solution:

- Ready to use Auramine Phenol contains 0.4g of Auramine O and 4g of Phenol.
- Cryptosporidium Differentiator (3% HCl) contains 3ml of Hydrochloric Acid and 97ml of Methanol.
- Ready to use Potassium Permanganate contains 0.1g Potassium Permanganate powder.

Concentrated Stains (dilute 1 part in 10 with deionised or reverse osmosed water before use):

-	PL.8008	Auramine Phenol	100ml
-	PL.8008/4.0	Auramine Phenol	400ml
-	PL.8008/5.0	Auramine Phenol	500ml
-	PL.8013	Potassium Permanganate	100ml
-	PL.8013/4.0	Potassium Permanganate	400ml
-	PL.8013/5.0	Potassium Permanganate	500ml

Per 100ml solution:

- Concentrated Auramine Phenol contains 4g of Auramine O and 40g of Phenol.
- Concentrated Potassium Permanganate contains 1g Potassium Permanganate powder.

MATERIALS REQUIRED BUT NOT PROVIDED

- Glass slides
- Inoculating loops
- Microscope
- Immersion Oil PL.396
- Pro-Slide™ Cryptosporidium Stain Control PL.4962

STABILITY AND STORAGE

The stains and differentiators should be stored at 15-25ºC in their original containers. Product stored under these conditions will be stable until the expiry date shown on the product label.

PRECAUTIONS

- For *In Vitro* Diagnostic Use only.
- For professional use only.
- Directions should be read and followed carefully.
- Do not use beyond the stated expiration dates.
- Microbial contamination may decrease the accuracy of the staining.
- Safety precautions should be taken in handling, processing and discarding all clinical specimens.
- Samples should be processed in the correct containment level conditions.
- Dispose of all material in accordance with local regulations.

TEST PROCEDURE

1. Prepare smear of material to be examined by emulsifying in saline on a clean glass slide and allow to air dry.
2. Place slide on a staining rack and fix in Cryptosporidium Fixative for 3 minutes. Allow to air dry.
3. Flood the slide with Auramine Phenol, stand for 10 minutes. Rinse with water.
4. Decolourise with Cryptosporidium differentiator (3% HCl) for 5 minutes. Rinse with water.
5. Flood the slide with Potassium Permanganate counterstain, stand for 30 seconds.
6. Rinse well with water. Allow to air dry or dry using gentle heat. Do not blot.
7. Examine using a fluorescent microscope.

QUALITY CONTROL PROCEDURE

Internal quality control of the stains and differentiators must be performed regularly on known reference material.

Recommended quality control:
Positive control – A proven positive
Negative control – A proven negative
Pro-Slide™ Cryptosporidium Stain Control PL.4962

INTERPRETATION OF RESULTS

Cryptosporidia oocysts will fluoresce bright green-yellow against a dark red background.

LIMITATIONS OF THE PROCEDURE

- Only experienced personnel should carry out the interpretation of stained slides.
- Read prepared slides as soon as possible after staining. Failure to do so may affect the results.
- False staining results can be seen due to cellular debris being stained by the technique.
- Organisms other than *Cryptosporidium* species may display varying degrees of acid-fastness, e.g. *Rhodococcus* species, *Mycobacteria* species, and *Isopora* species

REFERENCES

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- Kinyoun, J.J. 1915. A note on Uhlenhuth's method for sputum examination for tubercle bacilli. *American Journal of Clinical Pathology*. 46:472-4.
- Lennette. 1974. Manual of Clinical Microbiology. *American Society for Microbiology*, Washington, D.C.
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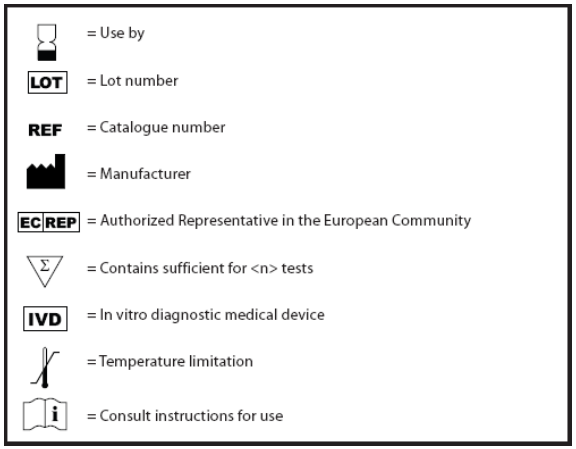

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HAZARDS IDENTIFICATION

Please refer to Safety Data sheets for full text for all hazard and precautionary statements.

 DANGER	PL.7072	H225, H302, H311+H331, H370 P210, P270, P280, P301+P310, P330, P304+P340, P311, P501
 DANGER	PL.7033/100 PL.7033/25 PL.7033 PL.7034 PL.7035	H314, H341, H412 P273, P280, P303+P361+P353, P305+P351+P338, P310, P501
 DANGER	PL.8008 PL.8008/4.0 PL.8008/5.0	H226, H301+H331, H312, H314, H341, H351, H373, H411 P210, P273, P280, P301+P330+P331, P310, P302+P352, P304+P340, P305+P351+P338, P501
	PL.7039/100 PL.7039/25 PL.7039 PL.7040	H412 P273, P501
	PL.8013 PL.8013/4.0 PL.8013/5.0	H411 P273, P391, P501
 DANGER	PL.7077	H311+H331, H301, H225, H319, H370 P210, P270, P280, P301+P310, P330, P303+P361+P353, P304+P340, P305+P351+P338, P312, P501

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