

LIGHT GREEN (For *Cryptosporidium* Acid-Fast Stain)

INTENDED USE

Remel Light Green is recommended for use as a counterstain in the modified acid-fast stain procedure for the detection of *Cryptosporidium* in clinical specimens.

SUMMARY AND EXPLANATION

Cryptosporidium is an intestinal protozoan parasite well known for causing diarrhea in animals and has more recently been recognized for causing human disease. The first case of human cryptosporidiosis was reported in 1976, and before 1982, only 7 cases of human cryptosporidiosis had been reported.¹ Since then, *Cryptosporidium* has emerged as an important cause of infections in immunocompromised hosts, especially those with AIDS (Acquired Immune Deficiency Syndrome).² In 1982, Current et al. reported 11 cases of human cryptosporidiosis in previously healthy persons who had known contact with infected calves.³ A modified acid-fast staining procedure was recommended by Ma and Soave because of its simplicity and specificity for detecting *Cryptosporidium*.⁴ A more recent modification of the Kinyoun carbol-fuchsin stain is recommended by Garcia for detection of *Cryptosporidium* oocysts in clinical specimens.⁵

PRINCIPLE

Cryptosporidium oocysts are spherical, 4-6 µm in diameter, and stain pink to red to purple with Kinyoun carbol-fuchsin stain. Most will contain several dark staining granules. Yeast cells, which are morphologically similar to *Cryptosporidium* oocysts, stain green.⁶

REAGENTS (CLASSICAL FORMULA)*

Light Green (CAS 5141-20-8).....2.0 g
Glacial Acetic Acid (CAS 64-19-7).....2.0 ml
Demineralized Water (CAS 7732-18-5).....1000.0 ml

*Adjusted as required to meet performance standards.

PRECAUTIONS

CAUTION! Possible cancer hazard; contains material which may cause cancer based on animal data. Avoid breathing vapor and eye/skin contact.

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and test materials after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

STORAGE

This product is ready for use and no further preparation is necessary. Store product in its original container at room temperature (20-25°C) until used.

PRODUCT DETERIORATION

This product should not be used if (1) the color has changed from green, (2) the expiration date has passed, or (3) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT

Specimens should be collected and handled following recommended guidelines. Use concentrated sediment of fresh or formalin-preserved stools. Other types of clinical specimens (e.g., duodenal fluid, bile, and most pulmonary sources) may also be used.²

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Collection containers, specimen preservative (Formalin 10%, REF R21640), (2) Centrifuge, (3) Applicator sticks, swabs, disposable pipettes, (4) Slide staining rack, forceps, (5) Glass microscope slides, coverslips, mounting medium, (6) Absolute Methanol (REF R40121), (7) TB Kinyoun Carbol-fuchsin (REF R40104), (8) Ethyl Alcohol 50%, (9) Sulfuric Acid 1% (REF R40124), (10) Quality control slides, (11) Microscope, immersion oil.

PROCEDURE

Every specimen represents a potential source of infectious material and should be handled accordingly.⁷

1. Make a thin smear of the specimen on a slide and allow it to air dry at room temperature.
2. Fix the smear with Absolute Methanol for 1 minute (dip slide).
3. Stain the slide with TB Kinyoun Carbol-fuchsin for 5 minutes at room temperature.
4. Wash slides with Ethyl Alcohol 50% (3-5 seconds) followed by an immediate wash with water.
5. Decolorize with Sulfuric Acid 1% for 2 minutes or until no more color runs from the slide. Rinse with water and drain.
6. Counterstain with Light Green for 1 minute. Rinse with water and drain.
7. Slides may be mounted with mounting medium and a coverslip.
8. Examine smear with bright field microscopy (40 X objective). Use oil immersion (100 X objective) to see internal morphology.

INTERPRETATION

Positive Test - Oocysts stain pink to red to purple, are 4-6 µm in diameter, and round or oval in shape. One to four sporozoites may be visible within the oocyst.

Negative Test - Yeast cells and background material stain green

QUALITY CONTROL

All lot numbers of Light Green have been tested and found to yield acceptable stain results. Quality control testing should be performed following procedures established by each laboratory according to applicable regulatory guidelines. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. With Kinyoun carbol-fuchsin, *Cryptosporidia* stain pink to red to purple depending on the stain penetration, the thickness of the smear, and the age of the specimen (length of time in fixative).⁶
2. Positive control slides should be tested prior to use of new lot numbers of permanent stain and at least weekly thereafter. If positive specimens are not available, use smears made from stool specimens containing leukocytes or epithelial cells to verify stain results.⁷
3. *Cryptosporidium* is more difficult to detect in formed stool specimens. Increasing the staining time has been reported to allow the oocysts to stand out from the background material.⁵
4. The hot acid-fast stain has been reported to maximize detection and identification of *Cryptosporidium* in formed stools.⁵

BIBLIOGRAPHY

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2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry, and M.A. Pfaller. 2007. Manual of Clinical Microbiology. 9th ed. ASM Press, Washington, D.C.
3. Current, E.L. and N.C. Reese. 1982. MMWR. 31:252-254.
4. Ma, P. and R. Soave. 1983. J. Infect. Dis. 147:824-828.
5. Garcia, L.S. 2001. Diagnostic Medical Parasitology. 4th ed. ASM Press, Washington, D.C.
6. Clinical and Laboratory Standards Institute (CLSI). 1997. Procedures for the Recovery and Identification of Parasites from the Intestinal Tract; Approved Guideline, M28-A. CLSI, Wayne, PA.
7. Isenberg, H.D. 2004. Clinical Microbiology Procedures Handbook. 2nd ed. ASM Press, Washington, D.C.

PACKAGING

REF R40123, Light Green 250 ml/Bottle

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

CAS (Chemical Abstracts Service Registry No.)

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