remel

DMSO MODIFIED ACID-FAST STAIN KIT

INTENDED USE
Remel DMSO Modified Acid-Fast Stain Kit is a rapid and permanent differential staining procedure for detection and identification of acid-fast organisms (i.e., *Cryptosporidium, Isospora,* and *Cyclospora* spp.) in fecal material. It is a modification of the Kinyoun method and may also be used for detecting acid-fast bacilli (*Mycobacterium* spp.) in sputum, urine, gastric lavage, other body fluids, and tissues.

SUMMARY AND EXPLANATION
Studies have shown *Cryptosporidium* is a significant enteric pathogen, capable of causing acute diarrhea in immunodeficient patients and transient diarrhea in immunocompetent individuals, particularly the very young.[1-5] *Isospora* has been recognized as a cause of human gastroenteritis for many years and in recent years, *Cyclospora* has been associated with outbreaks of diarrhea.[6] The need to facilitate appropriate treatment of infected patients has lead to the development of rapid methods for detection of these pathogens.

A variety of staining techniques have been described for detection of *Cryptosporidium* in fecal material, including: Giemsa, acridine orange, auramine-rhodamine, Kinyoun's, Ziehl-Neelsen, modified Ziehl-Neelsen, and negative stain with Kinyoun's carbolfuchsin.[7,8] Investigators have found the DMSO method as reliable and consistent in detecting cryptosporidiosis as more complicated test procedures. The method will also detect other pathogens, such as *Cyclospora* and *Isospora*, unlike some other staining methods.[9] It is faster and easier to perform and reduces the health risks associated with prolonged handling of contaminated material.

PRINCIPLE
Dimethylsulfoxide (DMSO) added to carbolfuchsin enhances penetration of acid-fast organisms by the stain and eliminates the need for heat or steam in the staining process. The stain is removed from background material by the decolorizing solution. Fast green counterstains the background material pale green. The oocysts of *Cryptosporidium, Cyclospora,* and *Isospora* stain pink to red to purple and are easily distinguishable against the pale-green background of the counterstain.

REAGENTS (CLASSICAL FORMULA)*

Reagent A Fuchsin-DMSO Stain:	2.5% Basic Fuchsin in a 45% solvent solution
Reagent B Decolorizer:	10% Sulfuric Acid solution
Reagent C Counterstain:	1.5% Fast Green Acid solution

*Adjusted as required to meet performance standards.

PRECAUTIONS
Reagent A: DANGER! Flammable liquid and vapor. Causes burns by all exposure routes. Harmful by inhalation, in contact with skin and if swallowed. Possible cancer hazard. May cause cancer based on animal data. Possible risks of irreversible effects. Substances known to cause developmental toxicity in humans.

Reagent B: DANGER! Causes burns by all exposure routes. Corrosive to metals.

Reagent C: WARNING! Causes eye, skin, and respiratory tract irritation.

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 media after use. Directions should be read and followed carefully. Refer to Material Safety Data Sheet for additional information on reagent chemicals.

MATERIALS SUPPLIED
Fuchsin-DMSO Stain (Reagent A)
Decolorizer (Reagent B)
Fast Green Counterstain (Reagent C)

STORAGE
Store product at room temperature. Container lids should be kept tightly closed. Reagent A, Fuchsin-DMSO, is light sensitive. Store in the dark when not in use.

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

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Coplin jars or staining jars with covers, (2) Microscope slides, (3) Absolute methanol, (4) Microscope with oil immersion lens, (5) Immersion oil, (6) Quality control slides.

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT
Specimens should be collected and handled following recommended guidelines.[10,11]

Preparation of Slides from Fecal Material:
Specimens should be collected and preserved in 10% formalin or SAF fixative for best results. Smears may be prepared from concentrated or unconcentrated material; however, concentration has been shown to provide a greater yield and facilitates detection of even small numbers of organisms.[10-12]

1. Unconcentrated fecal material fixed in preservative: Mix the specimen well. Spread a portion of the fixed specimen over a clean glass microscope slide, producing an uneven film with thick and thin areas. Allow the smear to air dry.
2. Fecal material concentrated by one of the following techniques:
    a. Formalin-Ethyl Acetate Sedimentation Technique:
        i. Follow procedure for concentration by sedimentation.
        ii. After straining the material through a filtration device or gauze, add saline and centrifuge as directed. **Note:** Filtration with gauze may result in entrapment of parasites.[11]
        iii. Decant supernatant, retaining sediment. Before adding the ethyl-acetate, prepare smear from the upper layer of the sediment. Spread a portion of the sediment over a clean glass slide, producing an uneven film with thick and thin areas. Allow the smear to air dry.
        iv. Continue concentration procedure as directed for other ova and parasites.
    b. Flotation Technique:
        i. Follow procedure for concentration of material by flotation.
        ii. After centrifugation, remove the material at the surface of the suspension with a wire loop.
        iii. Spread a portion of the material on a slide, producing thick and thin areas. Allow the smear to air dry.

Preparation of slides for *Mycobacterium* spp.:
1. Sputum: Transfer a loopful of specimen to a clean glass slide and spread to a thin smear. Allow the smear to air dry. **Note:** Specimen may be concentrated by centrifugation and the sediment used for the smear.
2. Urine and gastric lavage: Concentrate organisms by centrifugation. Prepare smears as above for sputum.
3. Tissue: Touch preparations are prepared according to histology procedure.

REAGENT PREPARATION

DMSO Stain reagents are ready to use. No additional preparation is required. Pour methanol and DMSO reagents into Coplin jars. Solutions should be changed every 30-40 slides.

PROCEDURE

1. Dip air-dried slides in absolute methanol for 10 seconds. Allow methanol to evaporate from the slide completely.
2. Place slides in **Reagent A** and stain for 5 minutes.
3. Rinse slides thoroughly with tap water and drain excess water.
4. Dip slides in **Reagent B** and decolorize for 5-10 seconds.
5. Rinse thoroughly with tap water and drain excess water.
6. Place slides in **Reagent C** and counterstain for 1-4 minutes.
7. Rinse thoroughly with tap water, drain excess, and allow slides to air dry.
8. Slides may be screened at 40 X for the presence of oocysts and sporozoites. Confirm results using the 100 X oil immersion lens.

NOTES

1. Longer staining times (up to several hours) in Reagent A have not been reported to be detrimental to stained smears.
2. Timing of steps 4 and 6 in the staining procedure will vary depending on the density of the smear.
3. Stained smears may be dehydrated and mounted with a coverslip for permanent documentation.
4. With experience, stained smears can be screened on low power (10 X) for pink *Cryptosporidium* oocysts.

INTERPRETATION OF THE TEST

1. *Cryptosporidium* oocysts are ovoid to spherical, 4-6 µm in diameter, and stain pink to red to deep purple. An internal vacuole and residual bodies that tend to clump to one side may be visible. Crescent-shaped sporozoites (up to 4) may be visible within the oocyst. Ghost cells (i.e., empty cyst walls) may be seen in specimens from a resolving infection. These structures are similar in size and shape to typical *Cryptosporidium* oocysts but with no visible internal structures. Some disintegrating, partially acid-fast particles may be seen within the oocyst.[11]
2. *Isospora* oocysts are 28-30 µm in diameter. The mature oocyst contains two sporocysts that measure 6-8 µm in size and stain pink to red. The oocyst wall does not stain.[11]
3. *Cyclospora* oocysts are round, 8-10 µm in diameter, and stain light-pink to deep red. Some oocysts contain granules or have a bubbly appearance. Oocysts that do not stain may have a wrinkled appearance.
4. Leukocytes and most yeast cells are not acid-fast and stain blue-green, though some yeast cells stain red with a dark overcast. Acid-fast bacteria stain pink to red, while non-acid-fast bacteria stain green. Vegetative cells, spores, and seeds may stain partially acid-fast with a brown or black overcast and a rough shell. Sperm capita stain pale-pink and are smaller than cryptosporidial oocysts.[6]

QUALITY CONTROL

All lot numbers of DMSO Modified Acid-Fast Stain have been tested and found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. To verify the efficacy of the reagents, include a positive control slide with each test run of specimens. Prepare a control slide from a 10% formalin-preserved fecal specimen containing *Cryptosporidium* oocysts. Degradation of staining characteristics in stained smears may be indicative of the need to freshen reagents in the jars.[11] If the cryptosporidia stain well, *Isospora* and *Cyclospora* oocysts can be expected to take up the stain, as well. If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

The DMSO stain may be used to detect the presence of acid-fast bacilli (*Mycobacterium* spp.) in respiratory specimens and other body fluids. Although a stained smear is not as sensitive as culture, smear examination provides for simple, rapid, presumptive identification of mycobacterial disease. Additional testing, including culture, is required for definitive identification of *Mycobacterium* spp. Consult appropriate references for further instructions.[10,11]

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PACKAGING

REF R246303, DMSO Stain Set..... 3 X 250 ml
REF R2463418, DMSO Reagent B (Decolorizer)..... 1 X 250 ml

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)

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