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| Giardia / Cryptosporidium FA Detection | | | | | | | | |
| **Purpose** | This procedure provides instruction for performing Cryptosporidium/Giardia FA Detection. | | | | | | | |
| **Principal and Clinical Significance** | The MeriFluor® Cryptosporidium/Giardia is a direct immunofluorescent detection procedure for the simultaneous detection *Cryptosporidium* oocysts and *Giardia* cysts in feces. The Detection Reagent contains FITC labeled monoclonal antibodies that bind to the cell wall antigens of *Cryptosporidium* and *Giardia* present in the specimen. Slides are prepared and stained with the Detection Reagent and Counterstain. The slides are then examined using a fluorescent microscope for the presence of apple-green fluorescence and characteristic morphology of *Cryptosporidium* oocysts and *Giardia* cysts. | | | | | | | |
| **Policy Statements** | This procedure applies to Microbiologists who perform Ova and Parasite exams. | | | | | | | |
| **Test Code** | CRID | | | | | | | |
| **Materials** |  | |  | | | |  |  |
|  | **Reagents** | | **Supplies** | | | | **Equipment** | |
|  | * MeriFluor® Cryptosporidium/Giardia kit: Meridian Bioscience product no. 250050  1. **Detection reagent**: FITC labeled anti-Cryptosporidium and anti-Giardia monoclonal antibodies 2. **Counterstain:** Eriochrome black 3. 20X wash buffer 4. Positive control 5. Negative control 6. Buffered glycerol mounting medium 7. Transfer loops 8. Treated slides   Do not mix reagents from different kit lot numbers.   * Para-Pak® Con-Trate® System: Meridian Bioscience product no. 960050  1. Con-Trate filter 2. Con-Trate disposable tube and cap 3. Reagent A (MucoPenX) 4. Reagent B (ethyl-acetate) | | * 10% Formalin * Distilled water * 0.9% Saline * Humidity chamber * 22 X 50 coverslips * Wooden applicator sticks * Wash bottle | | | | * Fluorescent microscope, excitation wavelength 490-500 * Centrifuge | |
| **Specimen** | 1. Acceptable specimens  * Stool specimens preserved in 10% formalin, SAF or ECOFIX® can be used. The specimen-preservative mixture must be allowed to stand for 30 min at RT for adequate fixation.  1. Unacceptable specimens  * Specimens preserved in PVA or MIF. * Specimens containing barium. Barium increases the background fluorescence, which interferes with readability.  1. SDES codes/Specimen type  * STO – stool * STO-ASP – stool aspirate * DA – duodenal aspirate  1. Refer to [Lab Test Directory–Cryptosporidium/Giardia FA](https://www.childrensmn.org/References/Lab/microbioviral/cryptosporidium-giardia-fa.pdf) for Specimen collection, transport and rejection criteria. | | | | | | | |
| **Special Safety Precautions** | Microbiologists are subject to occupational risks associated with specimen handling. Refer to the safety policies located in the safety section of the *Microbiology Procedure Manual***. Gloves must be worn during all phases of specimen processing.**   1. [*Biohazard Containment*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.1%20Biohazard%20Containment.docx) 2. [*Biohazardous Spills*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.4%20Biohazardous%20Spills.docx) 3. [*Safety in the Microbiology Laboratory*](file:///G:\Lab%20Procedures\Microbiology\1NEW%20Micro%20Procedure%20Manual.%20(same%20as%20in%20Starnet)\MCVI%203%20Safety\MCVI%203.2%20Safety%20in%20the%20Microbiology%20Lab.docx) | | | | | | | |
| **Quality Control** | 1. Positive: *Cryptosporidium* oocysts – slightly oval in shape, 2-6 µm in diameter; bright apple green.   *Giardia* cysts – oval shaped, 8-12 µm in diameter; bright apple green. Refer to Fig. 1.   1. Negative: No apple-green fluorescence. 2. Perform concurrent QC with each new lot or shipment before put into service by testing new reagents/controls in parallel with the new reagents/old controls. Record parallel testing results as entry #3 in the QC manual. 3. **Perform QC with each patient run**. Record results on daily QC board. 4. If there is a QC failure, document observation, notify supervisor and call Meridian Bioscience technical service at 1-800-343-3858 if necessary. | | | | | | | |
| **Storage** | 1. Store MeriFluor® C/G kit at 2-8ºC until expiration date. 2. Do not use kit components beyond the indicated expiration date. 3. Protect the Detection Reagent and Counterstain from exposure to light. 4. Allow coming to RT before use. 5. Return promptly to refrigerator after each use. 6. CAUTION: Do not freeze. | | | | | | | |
| **Reagent Preparation** | **1X Wash Buffer**   * 1. Prepare 100 ml of wash buffer from 20X buffer.   2. Add 5 ml 20X buffer to 95 mL of distilled water.   3. Store at RT up to 3 months. | | | | | | | |
| **Sample Preparation** | Unpreserved stool  1. Attach patient’s accession label to a sterile container. Use a white flat bottom 20mL container or 15 mL centrifuge tube. 2. Prepare a 1:3 to 1:5 dilution of stool to formalin using 5-10 grams of stool in 10-20 ml 10% formalin. 3. Use grape-sized amount of formed specimen with 20 ml 10% formalin. 4. Use 5 ml of liquid specimen with 10 ml 10% formalin. 5. Minimum amount: Add 3 grams stool to 6-12 mL10% formalin. 6. Mix contents thoroughly. Shaking for 1 min is usually sufficient. 7. Allow to stand at RT for 30 min to for adequate fixation. 8. Attach patient’s accession number to a coated CRID slide. 9. Using a transfer loop, place 20 µl of un-concentrated suspension onto the first well of a coated CRID slide. Spread the specimen over the entire well being careful not to scratch the treated surface.   **Para-Pak® Formalin transport system**   1. Formalin and PVA vials with sufficient stool added to each to bring the liquid level up to the “Fill to Here” line. 2. Mix contents thoroughly. 3. Allow to stand at RT for 30 min for adequate fixation. 4. Attach patient’s accession number to a coated CRID slide. 5. Using a transfer loop, place 20 µl of un-concentrated suspension onto the first well of a coated CRID slide. Spread the specimen over the entire well being careful not to scratch the treated surface.   **Duodenal aspirate**   1. If the specimen is ≥ 1 ml, centrifuge the specimen in a 15-ml centrifuge tube at 2000 rpm for 10 min. to concentrate the mucous and any organisms present. 2. Remove the supernatant leaving approximately 0.5 ml. 3. Mix well to resuspend the sediment. 4. Attach patient’s accession number to a coated CRID slide. 5. Prepare 2 wells on a coated CRID slide using a 20-µl loop. Spread the specimen over each entire well being careful not to scratch the treated surface.   **Specimen concentration**   1. Add 4 drops of reagent A to the Para-Pak® Formalin transport vial /10% formalin vial. Up to 8 drops can be added if the specimen is highly mucoid. 2. Close cap tightly and mix thoroughly by shaking the vial. 3. Label CON-Trate® 15ml centrifuge tube provided. 4. Insert a CON-Trate® filter into a 15 ml centrifuge tube (provided in kit). 5. Pour fecal suspension through the filter. DO NOT force through. Three ml is sufficient unless the suspension is thin. 6. Discard filter. 7. Add saline to the 12 ml mark. Cap tube and mix contents. 8. Centrifuge for 10 min at 2000 rpm. 9. Decant supernatant and retain sediment, approx. 1 ml. 10. Resuspend sediment in 10 ml of 10% formalin. 11. Add 3 ml of CON-Trate® B reagent, cap tube and shake for 30 sec. Invert the tube while shaking. Caution: Pressure may build up within the tube during shaking. Carefully release the pressure by opening the cap away from you. 12. Centrifuge for 10 min at 2000 rpm. 13. After centrifugation, the tube will have 4 layers from the top down:  * Layer consisting of Reagent B * Plug of fecal debris * Discolored aqueous layer * Sediment layer, containing the parasites. The final sediment should be 0.25-0.5 mL.  1. Hold tube at a 45º angle and free the plug of debris by ringing with a wooden applicator stick. 2. Decant supernatant into a satellite disposal flask for ethyl acetate, leaving the sediment. Do not turn the tube upright until the sides of the tube have been cleaned. 3. While holding the tube down, clean the sides of the tube with a cotton swab. 4. Using a transfer loop, place 20 µl of the concentrated suspension onto the second well of each previously prepared slide. Spread the specimen over the entire well being careful not to scratch the treated surface. 5. Prepare control slides with pos/neg in kit QC in the same manner on a separate slide. 6. Allow the slides to dry completely at RT (approx. 30 min). | | | | | | | |
| **Procedure** | 1. Place one drop of detection reagent in each well. 2. Place one drop of counterstain in each well. 3. Mix the reagents with an applicator stick and spread over the entire well. Do not scratch the surface of the wells. 4. Incubate the slides in a humidity chamber for 30 min at RT. **Protect from light.** 5. Using a wash bottle, rinse slides with a gentle stream of 1X buffer into the hazardous waste (mercury) container. Do not spray directly on the well to avoid disturbing the specimen. Prevent cross contamination by holding the long edge of the slide parallel to the hazardous waste (mercury) container. 6. Remove excess buffer by tapping the long edge of the slide on a paper towel. 7. Do not allow the slide to dry. 8. Add 1 drop of mounting medium to each well and coverslip. 9. Examine the slide using a fluorescence microscope at 100-200x. | | | | | | | |
| **Interpretation /Results** | 1. Positive:   *Cryptosporidium* oocysts – slightly oval in shape, 2-6 µm in diameter; bright apple green.  *Giardia* cysts – oval shaped, 8-12 µm in diameter; bright apple green.  A suture line may also be visible.     1. Negative: No apple-green fluorescence. 2. Background should counterstain dull orange to red. 3. Caution: If stool material is not seen upon scanning the slide wells, loss of specimen may have Crypptosporidium & Giardia IFAoccurred.   Figure 1 | | | | | | | |
| **Limitations** | 1. The presence of *Cryptosporidium* and *Giardia* often is associated with diarrhea and vomiting. However, shedding of oocysts and cysts has been observed in asymptomatic individuals. 2. The presence or absence of oocysts or cysts in a stool does not preclude the existence of other microorganisms or another underlying condition as the causative agent of a patient’s symptoms. | | | | | | | |
| **Method Performance Specifications** | 1. Loss of sample from slide well usually is due to overly vigorous washing or inadequate drying time. 2. Multiple specimens may need to be examined, since the number of oocysts/cysts may vary from day to day. A series of three specimens submitted on alternate days is recommended. 3. *Cryptosporidium* is a coccidian parasite of the intestines and respiratory tract of many animals including mice, sheep, snakes, turkeys, chickens, cows, monkeys, and domestic cats. 4. Although the organism is widely recognized as a disease of the immunocompromised patient, it can also cause disease in immunocompetent subjects. Animal contact, travel to endemic areas, living in a rural environment, day care attendance by toddlers, and exposure to contaminated public water have been recognized as risk factors for the development of cryptosporidiosis. 5. Children are more prone to develop infection than are adults. In these patients, the disease is a self-limited gastroenteritis, but in immunocompromised patients, a profound enteropathy results. A seasonal variation in incidence exists with the highest frequency reported in summer and autumn. 6. Giardiasis is one of the most common intestinal parasitic infections in the world. Contaminated food, untreated surface water polluted with cyst-containing animal feces in conjunction with inadequate filtration in water treatment facilities are the primary sources. In addition, public health authorities include Giardiasis as a sexually transmitted disease. | | | | | | | |
| **Result Reporting** | 1. Record results in Sunquest function MRE using the following MO codes:   PCOS: Positive: Cryptosporidium oocysts seen  NCOS: Negative: No Cryptosporidium oocysts seen  GLS : POSITIVE: Giardia lamblia cysts seen  NGLS : NEGATIVE: No Giardia lamblia cysts seen  CULTURE RESULTS  1. POSITIVE: Cryptosporidium oocysts present  2. NEGATIVE: No Giardia lamblia cysts seen    REPORT: FINAL 09272005 | | | | | | | |
| **References** | 1. Meridian Bioscience MeriFluor® Cryptosporidium/Giardia product insert SN 11220, April 2016, Meridian Bioscience, Inc. 3471 River Hills Drive, Cincinnati, Ohio, 45244. 2. Meridian Bioscience Para-Pak® CON-Trate® Stool Concentration Kit, product insert SN 10650, July 2014, Meridian Bioscience, Inc. 3471 River Hills Drive, Cincinnati, Ohio, 45244. | | | | | | | |
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| **Training Plan/ Competency Assessment** | **Training Plan** | | | | **Initial Competency Assessment** | | | |
| 1. Employee must read the procedure. 2. Employee will observe trainer performing the procedure. 3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer. | | | | 1. Direct observation. | | | |
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| **Historical Record** |  |  | |  | |  | | |
|  | **Version** | **Written/Revised by:** | | **Effective Date:** | | **Summary of Revisions** | | |
| 1.0 | Pat Ackerman | | 01/31/1992 | | Initial Version | | |
| 1.1 | Pat Ackerman | | 09/20/2005 | |  | | |
| 1.2 | Tina Gronquist | | 06/19/2014 | | Updated into online format | | |
|  | 2 | Becky Carlson | | 4/26/2015 | | Re-numbered from MC 502 for CMS load. | | |  |  |
| 2.1 | Becky Carlson | | 7/1/2015 | | Clarified Sample Preparation section. | | |
| 3 | Susan DeMeyere | | 5/8/2018 | | Biennial Review, update Logo | | |
|  | 4 | Susan DeMeyere | | 1/19/2024 | | Added to not mix reagents from different kit lot numbers. | | |
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