LABMOEBACK SU 2024

Procedures Manual

First Edition

DIAGNOSTICS of PARASITOLOGY LABORATORY

Title:

OVA and PARASITE TEST PROCEDURE

Principles:

An ova and parasite test to see if there is an intestinal parasite and its eggs (ova) by checking the sample of stool (poop) under a microscope. It is to see if intestinal parasites are causing symptoms that appear from an intestinal infection. The test helps diagnose parasitic infections that cause gastrointestinal symptoms such as diarrhea, abdominal pain, and cramping.

SPECIMEN REQUIREMENTS

COLLECTION:

Stool collection for parasite examination should be done using a clean, dry container or special paper or plastic wrap placed over the toilet to catch the stool. The lid should be securely closed, and parafilm or tape can be used to seal it. Fecal specimens must be collected in clean, wide-mouth containers with tight-fitting lids, ensuring no contamination with water or urine, as these can introduce elements that may be mistaken for parasites. Collection should always be performed before administering barium for radiologic exams.

REJECTING CRITERIA:
Stool specimens containing the opaque, chalky suspension are unacceptable, and intestinal protozoa may be masked for 5 to 10 days after ingestion of barium.

LABELING:
Label the container with the patient's name or (unique identifier), collection date, and time.

TRANSPORT:
Fresh stool specimens should be examined, processed, or preserved immediately, except when refrigeration is used, in which case they are suitable for antigen testing only. Preservation should occur as soon as possible. When transporting stool samples to the lab, they must be in leak-proof bags. If postal services are used, specimens must be packed according to national or international regulations, such as using the three-container approach and labeling with UN code 3373 for biological specimens.

REAGENTS OR MEDIA, SUPPLIES, EQUIPMENT

REAGENTS:
The fixatives and solutions include 10% formalin fixative in water, sodium acetate acetic acid formalin (SAF), merthiolate-iodine formalin (MIF), polyvinyl alcohol (PVA), Triton X-100 solution, and ethyl acetate.

SUPPLIES:
Essential laboratory supplies include various glassware such as graduated cylinders, flasks, bottles, funnels, centrifuge tubes, slides, coverslips, and pipettes. Other necessary items include sterile gauze, culture tube racks, applicator sticks, storage boxes, filter and lens paper, forceps, scissors, micrometers (stage and disk), and biohazard containers.

EQUIPMENT:
Laboratory equipment uses a general-use microscope, centrifuge, fume hood, and a class II-A1 or II-A2 biological safety cabinet (BSC).

STORAGE:
Refrigerator-freezer.

CALIBRATION

Calibration (If Applicable)
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Frequency: Parasite testing may be required frequently, especially in endemic areas or for symptomatic individuals. Routine testing may be performed annually or as needed based on exposure risk.

Step-wise Instructions in Parasite Testing:
1. Specimen Collection:
Collecting of stool samples using appropriate containers.
2. Specimen Processing:
-For fresh samples, process immediately to preserve the integrity of parasites.
-If needed, use fixation fluids like formalin or saline for preservation.
-stool samples should use direct smears or concentration techniques to identify helminth eggs, larvae, or adult forms.
3. Staining and Examination:
Apply appropriate staining (e.g., trichrome or iron-hematoxylin) for protozoa detection.
For blood-borne parasites (e.g., Plasmodium), collect and smear blood, followed by staining (Giemsa) and microscopic examination.

QUALITY CONTROL

CONTROL MATERIAL:

Positive Control Samples: Known samples containing specific parasites to verify that the testing method can detect them.
Negative Control Samples: Samples that do not contain any parasites to ensure that the test does not produce false positives.

PREPARATION/HANDLING:

Preparation: Control materials should be prepared according to standardized protocols to ensure consistency. This may involve diluting samples to specific concentrations.
Handling: Use gloves and proper aseptic techniques to avoid contamination.
Storage: Store control materials at appropriate temperatures (usually refrigerated) and protect them from light and contamination. Label them clearly with the dates collected.

FREQUENCY OF TESTING:

Routine Testing: Depending on the test volume, QC materials should be tested with each batch of patient samples or at regular intervals (e.g., daily, weekly) after Equipment Calibration.
Perform QC testing after any maintenance or calibration of laboratory equipment.

EXPECTED RESULTS:

Positive Controls: It should yield positive results for the specific parasites they detect.
Negative Controls: It Should yield negative results, confirming that no contamination or false positives occur.

CORRECTIVE ACTIONS:

If control results do not meet expectations, the following steps should be taken: investigate the cause of failure, such as reagent issues or procedural errors; re-test using fresh controls; review procedures to ensure all protocols are correctly followed; and document any deviations along with the actions taken to resolve the issues.

RECORDING AND STORAGE OF QC DATA:

Maintain a log of all QC results, including dates, personnel involved, and any corrective actions taken.

ALTERNATIVES :

Previously tested samples can serve as controls if available.

STEP-BY-STEP INSTRUCTIONS

Microscopic Examination (Direct et al.)

Step 1: Prepare a direct wet smear by mixing a small amount of fresh, unpreserved stool with 0.85% saline.
Step 2: Place a 22 x 22-mm coverslip over the suspension and examine the sample under the microscope using the low-power (10×) objective.
Step 3: Use low light intensity and Kohler illumination to detect motile protozoan trophozoites.
Step 4: If necessary, switch to 40× magnification for closer inspection of suspicious objects.
Step 5: Optional: Add iodine at the edge of the coverslip for color contrast (note that this will stop motility). If the stool has been preserved, omit this step, as motility cannot be observed.

Microscopic Examination (Concentration Procedures)
Step 1: Concentrate the stool specimen using one of two methods: sedimentation or flotation.
Step 2: For sedimentation (formalin-ethyl acetate method), strain the stool specimen through gauze into a conical centrifuge tube and mix with saline or formalin.
Step 3: Centrifuge the mixture at 500 × g for at least 10 minutes, then add ethyl acetate to separate debris and fat from the parasites, which will remain in the sediment.
Step 4: Decant the supernatant, resuspend the sediment with saline or formalin, and examine the sediment as a wet preparation.

REPORTING RESULTS

1. **Format of Report**:
    - Include patient identification, date of sample collection, and date of report.
    - Clearly state the results for each sample, indicating whether parasites were detected and specifying the type of parasites identified.
2. **Result Categories**:
    - **Positive**: Indicate the presence of specific parasites (e.g., "Positive for Giardia lamblia").
    - **Negative**: State that no parasites were detected.
    - **Inconclusive**: Further testing or re-sampling is recommended if results need to be clarified.
3. **Statistical Analysis**:
    - Include any statistical analysis performed, such as sensitivity, specificity, and concordance rates between ParaTechs and MedParas.
4. **Interpretation**:
    - Provide a brief interpretation of the results, including potential clinical implications and recommendations for further action or treatment.

Procedure Notes

1. **Sample Collection**:
    - Ensure samples are collected in clean, labeled containers and transported under appropriate conditions (e.g., refrigerated if necessary).

2. **Sample Processing**:
    - Follow standardized protocols for processing samples, including homogenization, dilution, and sedimentation.
    - Use appropriate techniques for microscopic examination (e.g., wet mount, iodine staining).
3. **Quality Control**:
    - Implement QC measures as previously described, including testing control and patient samples.
4. **Documentation**:
    - Maintain detailed records of all procedures, including deviations from standard protocols, results of QC tests, and any corrective actions taken.
5. **Ethical Considerations**:
    - Ensure that all participants provide informed consent and that data confidentiality is maintained throughout the testing process.
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Limitations of Methods

Several factors affect the accuracy of parasite testing. (Sensitivity and specificity) are crucial; low sensitivity can lead to false negatives, especially in low parasite loads, while low specificity may result in false positives from misidentified non-parasitic elements. (Sample quality) is vital, as contamination and improper storage can compromise results. Laboratory personnel's (technical expertise) is also essential, as inadequate training can lead to misinterpretation. (Equipment limitations) may hinder proper testing, and some methods may have a (limited detection range), missing certain parasites. Additionally, (environmental factors) like temperature and humidity can impact parasite viability, and a lack of (quality control) measures can result in undetected errors, leading to unreliable results.

Troubleshooting or Backup Plan

If initial results are inconclusive or unexpected, consider re-sampling the patient under optimal conditions to confirm findings. Additionally, alternative diagnostic methods, such as molecular techniques (PCR) or serological tests, should be utilized when traditional methods yield ambiguous results. Regular quality control checks on reagents, equipment, and procedures should be performed, and if QC results are unsatisfactory, investigate and rectify any issues before processing patient samples.

REFERENCES:

Lou J, Yu Y, Dai F. Laboratory Test for Diagnosis of Parasitic Diseases. Radiology of Parasitic Diseases. 2016. Practical Guidance for Clinical Microbiology Laboratories: Laboratory Diagnosis of Parasites from the Gastrointestinal Tract. Clin Microbiol Rev. 2017

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SIGNATURE OF LABORATORY DIRECTOR
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