Despite recent advances in diagnostic technology, microscopic examination of stool specimens remains central to the diagnosis of most pathogenic intestinal protozoa.

1-*Giardia lamblia*

2-G. duodenalis

3-Cryptosporidium spp.

4-Dientamoeba fragilis

5- Entamoeba histolytica

6-Cyclospora cayetanensis.

*Protozoan infections significantly contribute to the burden of gastrointestinal illness worldwide.

*The microscopic ova and parasite examination (O&P) is the traditional method for stool parasite testing.

The clinical laboratory challenges in the detection of intestinal protozoa:

1. Reliance on labor-intensive, technically demanding tests (e.g., O&P)

· O&P testing is left until other laboratory testing is completed, yielding long turnaround times, due to the misguided notion this testing is "less critical" than others

· Many laboratories do not have technologists that can reliably identify pathogens and differentiate these from nonpathogenic species or artifacts

2. Reliance on insensitive tests

· O&P is associated with a sensitivity of 20 to 90% compared to molecular assays

· Some antigen detection tests, e.g., those for Cryptosporidium spp., are insensitive

3. Shortage of clinical specimens positive for intestinal protozoa

· Limits the opportunities for adequate training, Limits ability of technologists to maintain proficiency

4. Shortage of training programs/resources for parasitology

· Confounded by the retirement of experienced technologists who would otherwise perform training

5. Suboptimal physician ordering practices

· Few physicians will order organism-specific tests, even during outbreaks

- Sensitivities and specificities of FDA-approved assays for molecular and serologic detection of intestinal protozoan parasites

Entamoeba histolytica

1-Stool enzyme immunoassay

2-Serum-based EIAs

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Entamoeba dispar

1-Stool enzyme immunoassay

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Giardia lamblia

1-Stool enzyme immunoassay

2-Immunochromatography

3-Direct fluorescent antibody

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Cryptosporidium spp.

1-Stool enzyme immunoassay

2-Direct fluorescent antibody

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Cryptosporidium spp. and ***Giardia intestinalisc***

1-Stool enzyme immunoassay

2-Immunochromatography

3-Direct fluorescent antibody

4-Multiplex PCR

SPECIMEN COLLECTION

1-Optimal recovery and microscopic identification of protozoa from patients with intestinal infections is dependent on proper collection and preservation of fecal specimens.

2-Well-recognized factors that influence the sensitivity of parasite examinations include patient medications, specimen collection interval, and the preservation of stool prior to testing. The diagnostic yield of the O&P is also significantly impacted by the number of stool specimens collected and submitted to the laboratory for testing. Many intestinal protozoa are irregularly shed, and data suggest that a single stool specimen submitted for microscopic examination will detect 58 to 72% of protozoa present.

3- alternative approaches have been proposed to help curtail unnecessary testing, including application of an algorithm that requires a negative specimen and persistence of symptoms before a second or third specimen is analyzed by the laboratory.

4-Specimens may also be pooled prior to screening based on microscopy. In contrast, the enhanced sensitivity of molecular detection methods may require only 1 specimen for testing to achieve sensitivity equal to, if not greater than, microscopy.

STOOL PRESERVATION

While visualization of motility in unpreserved specimens may facilitate diagnosis, this technique is impractical for most laboratories, as transport of fresh stool to the laboratory for testing is rarely within the requisite time frame for examination (i.e., 30 to 60 min).

A variety of stool fixatives have been developed and modified in recent decades for use with traditional microscopic examination:

1-formalin

2-sodium acetate-acetic acid-formalin (SAF)

3-Schaudinn's fluid

4-polyvinyl alcohol-containing fixatives (mercury, copper, or zinc based), and mercury-free/formalin-free fixative.

Alternative stool preservatives.

Zinc- and copper-based polyvinyl alcohol (PVA) formulations have been developed and are commercially available to replace the mercury-based fixatives