Laboratory diagnosis of enteric protozoa

Protozoa are unicellular organisms classified as eukaryotes. Protozoa responsible for intestinal infections are *Entamoeba*, *Giardia*, *Cryptosporidium*, *Cyclospora*, *Cystoisospora*, *Dientamoeba*, *Blastocystis*, and *Balantidium*. These enteric protozoa are associated with diarrheal illnesses in humans, with some causing severe debilitating illnesses, especially in immunosuppressed populations. The diagnosis of these enteric protozoa has traditionally been challenging.

1- Microscopy: For many years, microscopy has been the only tool available for the detection of parasites through inspection of feces, blood smears , tissue specimens, , lymph node aspirates, bone marrow , and even cerebrospinal fluid . However, sample preparation for direct observation is time-consuming, labour intensive, and proper diagnosis depends on qualified laboratory technicians. In the case of slide reading, a second independent reading is preferable, but not always required for accurate diagnosis. If need be, divided readings are resolved by a third reader. In endemic regions, where resources are limited, this proves to be difficult and misdiagnosis can significantly impact patient care.
In reality, all major intestinal infections are still solely dependent on microscopy for diagnosis. As for other parasite infections, many are confirmed by the use of microscopy in conjunction to other methods of diagnosis including serology-based assays and more recently molecular-based assays.

2- In situations where biologic samples or tissue specimens are unavailable, serology alone is the gold standard for diagnosis. Serology-based diagnosis tools can be divided into two categories: antigen-detection assays and antibody-detection assays. These include the enzyme-linked immunosorbent assay (ELISA), also called enzyme immunoassay (EIA), and all its derived tests such as the Falcon assay screening test ELISA (FAST-ELISA) and the dot-ELISA. Other assays include the hemagglutination (HA) test, indirect or direct immunofluorescent antibody (IFA or DFA) tests, complement fixation (CF) test, and immunoblotting and rapid diagnostic tests (RDTs).

3-Dipstick. Point of care tests to detect protozoa would be appropriate technology for the developing world. There are at least two such tests that are in the early stages of development.

4-Rapid Antigen Detection. Antigen detection methods can be performed quickly and do not require an experienced and skilled morphologist. Much work has been accomplished on the development of antigen detection tests, resulting in commercially available reagents for the intestinal parasites *Cryptosporidium* spp., *Entamoeba histolytica*, *Giardia duodenalis*, and *Trichomonas vaginalis*. In addition, antigen detection tests using blood or serum are available for *Plasmodium* and *Wuchereria bancrofti*.

Rapid antigen detection tests (RDTs) based on immunochromatographic antigen detection have been implemented in many diagnostic laboratories as an adjunct to microscopy for the diagnosis of malaria. RDTs consist of capturing soluble proteins by complexing them with capture antibodies embedded on a nitrocellulose strip. A drop of blood sample is applied to the strip and eluted from the nitrocellulose strip by the addition of a few drops of buffer containing a labeled antibody. The antigen-antibody complex can then be visualized directly from the membrane.

5- Molecular-Based Approaches

Nucleic Acid-Based Approaches

The many limitations of microscopy and serology-based assays have influenced parasitologists towards the use of gene amplification methods made possible with the advent of the polymerase chain reaction (PCR). Besides the traditional PCR, including nested and multiplexed PCR, the real-time PCR (RT-PCR) is implemented for the detection of several parasitic infections. Newer technologies such as loop-mediated isothermal amplification and Luminex-based assays have also emerged as possible new approaches for the diagnosis of parasitic diseases.

Molecular-based approaches based on nucleic acids offer greater sensitivity and specificity over the existing diagnostic tests. They permit the detection of infections from very low parasitized samples including those from asymptomatic patient's samples. Moreover, multiplexed PCR allows for the detection of multiple sequences in the same reaction tube proving useful in the diagnosis of several parasitic infections.

Real-Time Polymerase Chain Reaction (RT-PCR)

RT-PCR system unlike conventional PCR, allow for the quantification of the original template's concentration through the use of various fluorescent chemistries, such as Sybergreen, Taqman probes, fluorescence resonance energy transfer (FRET), and Scorpion primers . The concentration is measured through comparison to standard curves. This eliminates the need to visualize the amplicons by gel electrophoresis

thereby greatly reducing the risk of contamination and the introduction of false-positives. When multiplexed, RT-PCR allows for the high-throughput analysis of different sequences in one single-closed tube reaction.

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a unique amplification method with extremely high specificity and sensitivity able to discriminate between a single nucleotide difference. It is characterised by the use of six different primers specifically designed to recognise eight distinct regions on a target gene, with amplification only occurring if all primers bind and form a product.

Luminex xMAP Technology

Luminex technology is a bead-based flow-cytometric assay that allows the detection of various targets simultaneously. The microsphere beads can be covalently bound to antigens, antibodies, or oligonucleotides that will serve as probes in the assay. Up to 100 microspheres are available each emitting unique fluorescent signals when excited by laser therefore allowing the identification of different targets. Adapted to the study of parasites, the Luminex assay could identify multiple organisms or different genotypes of one particular organism during the same reaction utilizing very low volume. The approach could prove useful in the study of antigenic diversity and drug-resistance alleles and for the diagnosis of parasitic diseases.

Proteomics

Since proteins are the main catalysts, structural elements, signaling messengers, and molecular machines of biological tissues, proteomic studies are able to provide substantial clinical relevance. Proteins can be utilized as biomarkers for tissues, cell types, developmental stages, and disease states as well as potential targets for drug discovery and interventional approaches. The next generation of diagnostic tests for infectious diseases will emerge from proteomic studies of serum and other body fluids. Recent advances in this area are attributable largely to the introduction of mass spectrometry platforms capable of screening complex biological fluids for individual protein and peptide “biomarkers.” Proteomic strategy can identify proteins in two ways: bottom-up and top-down approaches.