Molecular Diagnostics and Parasitic Disease

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KEYWORDS

• Malaria • PCR • *Trichomonas* • Leishmaniasis • Babesiosis • Trypanosomiasis • Amebiasis • NAAT

KEY POINTS

- Molecular tests play a growing role as adjuncts to traditional parasitology diagnostics, and in select situations, may replace traditional methods.
- Benefits of molecular methods may include increased sensitivity and specificity, but standardization of assays and paucity of commercial platforms are major limitations.
- Instrumentation and work flow requirements pose significant challenges for many parasite-endemic, resource-poor settings, although new applications of isothermal methods show significant promise for wider implementation.

INTRODUCTION

Despite advances in medical knowledge and practice, parasitic diseases remain a significant global health burden. Malaria alone is estimated to have caused 216 million infections in 2010.[1] In addition to malaria, 11 of the 17 'neglected tropical diseases' identified by the World Health Organization (WHO), which affect 1 billion persons overall, are parasitic in origin.[2] Although often thought of as an affliction of residents of tropical and developing countries and travelers, these parasitic diseases cause significant morbidity in developed countries too. In the United States, for example, the Centers for Disease Control and Prevention (CDC) has identified 5 parasitic diseases (Chagas disease, neurocysticercosis, toxocariasis, toxoplasmosis, and trichomoniasis) that require public health action based on their substantial national disease burden.

Diagnostics in parasitology have traditionally centered on morphology using light microscopy and various histochemical stains. Although this is a time-honored and valuable technique, morphologic interpretation is subjective and requires significant expertise. With the advent of polymerase chain reaction (PCR) testing in the 1980s, molecular assays have been developed for most parasitic human infections, including

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infections with significant worldwide morbidity and mortality, such as malaria and leishmaniasis. Since then, developments in postamplification techniques (eg, microarrays, DNA sequencing), and also recent advances in mass spectrometry (MS) and proteomics, have shown promise for the laboratory diagnosis of parasitic infections. Regardless of these advances, most molecular tests are not well suited for widespread adoption in resource-poor/field settings, in which many of these diseases are endemic. Testing systems are often complex and expensive, requiring sophisticated instrumentation, molecular grade reagents, highly skilled operators, consistent electricity sources, temperature and humidity controls, and highly regulated transportation and storage capabilities for patient specimens and reagents (ie, maintenance of a cold chain). New applications of isothermal nucleic acid amplification tests (NAAT) such as loop-mediated isothermal amplification (LAMP) and nucleic acid sequence based amplification (NASBA) show promise for future widespread implementation in resource-poor settings because need for a thermocycler is obviated, although additional challenges remain.

The paucity of commercially available and US Food and Drug Administration (FDA)-approved/CE-marked molecular parasitology tests is also problematic. Most molecular tests are based on nonstandardized, laboratory-developed methods, requiring significant maintenance demands and quality control measures to ensure optimal assay performance. As a result, the use of laboratory-developed tests is generally limited to centralized reference laboratories, public health laboratories such as the CDC, and specialized research facilities. Several new methods are under development and are expected to be commercially available in the future.

As the use of molecular methods become more widespread, clinicians and laboratorians need to address important questions such as how the tests should be incorporated into the work flow of the parasitology laboratory, the role of traditional diagnostics as supplemental or confirmatory methods, and the interpretation of positive results with respect to clinical infection. In particular, molecular testing is often more sensitive than traditional microscopy, and nucleic acid may be detectable long after the patient has been successfully treated. In these situations, it is not well understood if individuals with positive molecular tests invariably progress to clinical disease or experience a relapse after treatment.

Despite these uncertainties, we believe that in the near future, molecular diagnostics for parasitology will become more accessible and standardized for use, both in the field and in clinical laboratories for screening diagnosis of parasitic infections. In this review, the available molecular assays for the diagnosis of the major and more common human parasites are examined (see also **Table 1**). These assays include the few that have been approved or cleared by the FDA, as well as the more widespread laboratory-developed tests. Newer technologies such as isothermal amplification techniques and MS as applied to diagnostic molecular parasitology are also briefly reviewed.

BLOOD PARASITES

Malaria

Background

Malaria is a potentially deadly infection caused by protozoan parasites in the *Plasmodium* genus. Infection is transmitted by the bite of an infected female *Anopheles* sp mosquito, resulting in erythrocyte infection and destruction. Although once widespread, disease is now mostly limited to the tropics and subtropics worldwide, including many poor nations with limited resources and health care infrastructure.