These protocols have now been optimized and can potentially be applied widely in the majority of solid tumors including breast, lung, colon, ovary and others malignacies.

Techniques employed in genetic engineering will be able to provide chimeric proteins made up of antibody fragments and recombinant avidin in order to obtain an antibody molecule conjugated with a modified avidin tetramer of low immunogenicity.

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A NONRADIOACTIVE, BRANCHED DNA-BASED TECHNIQUE FOR DETECTION OF TRYPANOSOMA BRUCEI SPP. IN BLOOD

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INTRODUCTION

Since their recent advent, molecular diagnostic methodologies have proven to be very useful and offer a number of advantages over microscopic, biochemical, and immunologic procedures for the detection of pathogens. To date, target amplification methods such as the polymerase chain reaction (PCR) have been the most commonly applied molecular techniques. The branched DNA (bDNA) Signal Amplification Assay is an alternative hybridization based system for the sensitive and rapid detection of agents of infectious disease. This molecular technique amplifies the signal from a target molecule rather than the target itself, and thus avoids artifactual problems that have hampered other molecular diagnostic methodologies (1).

We have developed a nonradioactive bDNA-based assay for detection of *Trypanosoma brucei* in clinical samples. *T. brucei gambiense* and *T. brucei rhodesiense* are the etiologic agents of sleeping sickness, and the disease is considered a major health problem in many African countries for humans as well as for cattle, which are infected with *T. brucei.* The accurate diagnosis of African sleeping sickness by direct blood examination is problematic due to the wave-like fluctuations in levels of parasites present in biological samples, while immunologic tests are hampered by the trademark antigenic variation of *T. brucei* used by the parasite to avoid the host immune response. We have developed a sensitive and specific diagnostic assay for African trypanosomiasis, regardless of the stage of infection, variable antigen type (VAT), or subspecies of *T. brucei.*

MATERIALS AND METHODS

In the branched DNA assay, crude lysates of samples are denatured and hybridized in solution to two sets of oligonucleotide probes (50 mers). One set serves to capture the target sequence from the organism of interest by hybridizing to both the target sequence and oligonucleotide probes bound to microtiter dish wells. The other set of probes contains regions complementary to both the target sequence and to branched DNA molecules and serves to amplify the signal. Each of the 45 branches in this amplifier structure then hybridizes to an alkaline phosphatase-labeled probe. Finally, the complex is detected by the addition of an enzyme-triggerable chemiluminescent substrate, and light emission is measured

with a luminometer or a Polaroid camera (1). Plasma and Buffy coat were prepared from heparinized blood samples and used directly in the *T. brucei* bDNA assay (2).

RESULTS AND DISCUSSION

Two repetitive DNA sequences specific to the *Trypanosoma brucei* complex were chosen as targets, namely the 177 bp satellite repeat (3) and the RIME sequences (4, 5), and the appropriate oligonucleotide probes were designed and tested. Parasites were detected in biological samples with clinically relevant sensitivity (5-10 parasites/μL of blood), and comparable limits of detection were observed with cloned target sequences, purified *T. brucei* DNA, procyclic trypanosomes and bloodstream trypomastigotes. Analysis of the species specificity of the *T. brucei* bDNA assay with serial dilutions of purified DNAs revealed a strong signal from the three *T. brucei* subspecies and no signal from a variety of related organisms.

Thus, the branched DNA technology offers certain advantages over alternative molecular techniques, including the simplicity of sample preparation and of the procedure itself, the stability of the reagents, the ability to process large numbers of samples simultaneously, and freedom from cross-contamination artifacts.

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INTRODUCTION AND IMPLEMENTATION OF MOLECULAR TECHNOLOGY FOR THE DIAGNOSIS AND EPIDEMIOLOGY OF INFECTIOUS DISEASES IN LATIN AMERICA

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INTRODUCTION

Molecular techniques, such as the polymerase chain reaction (PCR) and nonradioactive DNA probes, can be applied to the diagnosis and epidemiology of infectious diseases in countries of limited resources. When appropriately implemented, these molecular techniques are more rapid, more sensitive, more specific, safer, and less costly than existing methods. We have developed a successful format for the transfer of molecular technology to Latin American countries through the use of on-site hands-on workshops (1).

The program is comprised of sequentially staged and progressively more complex courses which provide participants with solid experience in the theory and practice of molecular technology, epidemiology and proposal development. The courses progress from the introduction of molecular technology (Phase I) to its implementation by local scientists (Phase II and beyond) and are accessible to a wide range of participants, since prior training is not a pre-requisite. Course I is designed in consultation with local scientists, who select the pathogens to be detected based on national health priorities. Participants in the first workshop then design the pilot studies they will conduct in Course II and collect the appropriate samples. These pilot studies from the basis of grant proposals detailing larger studies which are developed during Course II. These courses have been conducted in Nicaragua and Ecuador and are planned in a number of additional countries in the region.

MATERIALS AND METHODS

Existing methodologies (PCR and nonradioactive DNA probes) for detection of a range of pathogens were adapted for country-specific applications, allowing course participants to detect *Leishmania, V. cholerae, M. tuberculosis*, dengue virus, *Shigella* and enterotoxigenic *E. coli*, and *P. falciparum* in clinical and environmental samples. Extraction procedures were simplified for rapid processing, and PCR was carried out by manual amplification or with a thermocycler, if available. Simple but effective precautions were used to minimize the chance of cross-contamination of samples, including fre-